SN 09/760,949 (BS)

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : C07K 7/56, 7/08, A61K 38/10, 38/12 — A1

(11) International Publication Number: **WO 00/05251**

(43) International Publication Date: 3 February 2000 (03.02.00)

(21) International Application Number: PCT/EP99/05235

(22) International Filing Date: 22 July 1999 (22.07.99)

(30) Priority Data:

98113744.1	23 July 1998 (23.07.98)	EP
99107637.3	16 April 1999 (16.04.99)	EP

(71) Applicant: F. HOFFMANN–LA ROCHE AG [CH/CH]; 124 Grenzacherstrasse, CH–4070 Basle (CH).

(72) Inventors: AOKI, Masahiro; Kowada 3–6–2, Chigasaki–shi, Kanagawa–ken (JP). KOHCHI, Masami; Takakura 509–1–D–202, Fujisawa–shi, Kanagawa–ken (JP). MASUBUCHI, Kazunao; Naganuma–cho 191–1–406, Sakae–ku, Yokohama–shi, Kanagawa–ken (JP). MIZUGUCHI, Eisaku; Shiromeguri 690–203, Kamakura–shi, Kanagawa–ken (JP). MURATA, Takeshi; Higashikaigan–Minami 6–6–17–I–106, Chigasaki–shi, Kanagawa–ken (JP). OHKUMA, Hiroaki; Inaridai 3–19, Itabashi–ku (JP). OKADA, Takehiro; Fujigatani 4–17–17–102, Kugenuma, Fujisawa–shi, Kanagawa–ken (JP). SAKAITANI, Masahiro; Kowada 3–8–12–1002, Chigasaki–shi, Kanagawa–ken (JP). SHIMMA, Nobuo; Higashikaigan–Minami 2–11–19, Chigasaki–shi, Kanagawa–ken (JP). WATANABE, Takahide; Imaizumidai 6–26–3, Kamakura–shi, Kanagawa–ken (JP). YANAGISAWA, Mieko; Splingsplaza–Izumino 206, Izumi–cho 5626–2, Izumi–ku, Yokohama–shi, Kanagawa–ken (JP). YASUDA, Yuri; Mezonpeach II–201, Matsubayashi 2–12–35, Chigasaki–shi, Kanagawa–ken (JP).

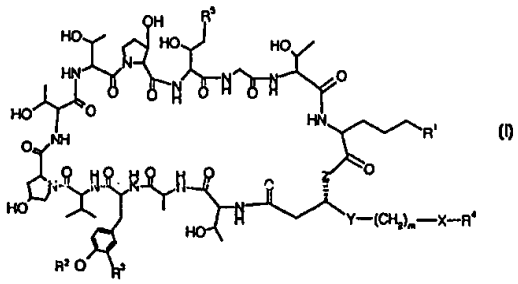
(74) Agent: WITTE, Hubert; 124 Grenzacherstrasse, CH–4070 Basle (CH).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

With international search report.

(54) Title: AEROTHRICIN ANALOGS, THEIR PREPARATION AND USE



(I)

(57) Abstract

The present invention relates to novel Aerothricins represented by Formula (I), wherein R^1, R^2, R^3, R^4, R^5, X, Y, Z, and m are as defined in Claim 1; and pharmaceutically acceptable salts thereof. The present invention also relates to a pharmaceutical composition comprising an Aerothricin of Formula (I) and a pharmaceutically acceptable carrier. Furthermore, the present invention relates to the use of such Aerothricins for the preparation of medicaments, as well as to processes and intermediates for the preparation of the Aerothricins of Formula (I).

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

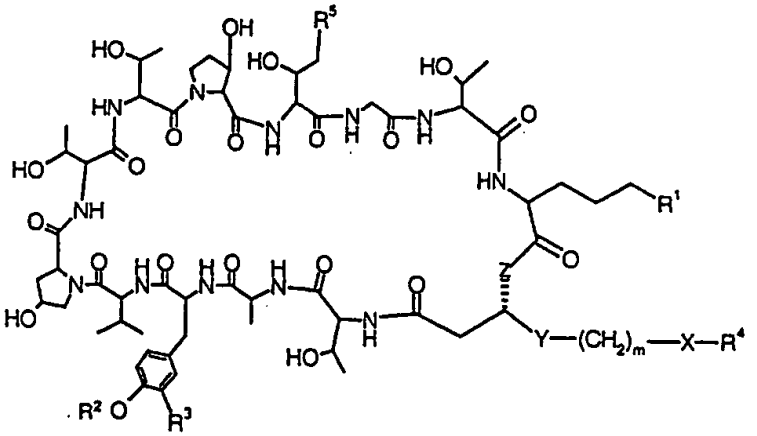
AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

AEROTHRICIN ANALOGS, THEIR PREPARATION AND USE

The present invention relates to novel cyclic compounds having antifungal activity (hereinafter referred to as Aerothricins), the use of Aerothricins in the medical therapy, pharmaceutical compositions containing Aerothricins as well as to processes and intermediates for the preparation of Aerothricins.

Azole antifungal agents are currently widely used for the treatment of systemic mycoses. However, long term prophylactic use of azole antifungals resulted in generation of azole resistant *Candida spp.* due to their fungistatic action. Therefore, fungicidal agents are particularly important for treatment of severe systemic mycoses. Furthermore, the currently available antifungal agents are not effective against *Fusarium spp.* which is one of the emerging pathogens among immunocompromised patients. Amphotericin B is a highly effective fungicidal agent currently used clinically, but its therapeutic index (effective dose vs. toxic dose) is rather narrow. Certain cyclic compounds such as LY303366 (EP 736 541), WF11243 (EP 584 360) are known to show fungicidal activity through inhibition of β-1,3-glucan synthase. However, they have still some disadvantages in terms of antifungal spectrum and/or safety profile. Thus, development of new fungicidal agents with better safety profile and efficacy against major systemic pathogens including newly emerging pathogens like *Fusarium spp.* is urgently required.

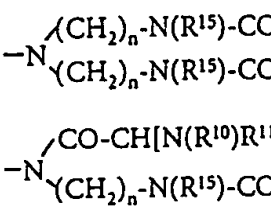
In particular, the present invention relates to novel Aerothricins represented by the Formula (I),



(I)

wherein

R^1 is guanidino, tri-lower alkylammonio, $-N(R^{10})-R^{11}$, $-N(R^{15})-CO-R^{14}$, $-N(R^{15})-CO-CH[N(R^{10})R^{11}]-R^{13}$, $-NHCOCH(R^{13})-NHCOCH(NH_2)-R^{13}$,



;

R^{10} and R^{11} are each independently selected from hydrogen; heteroaryl substituted with one or two amino; lower alkyl optionally substituted with one or more, preferably one or two, amino, amino-lower alkyl, cyano, guanidino, nitrogen containing heterocycle(s) or phenyl group(s) containing an amino, amidino or guanidino group;

R^{13} is a residue derived from natural or unnatural amino acids;

R^{14} is lower alkyl substituted with one or more, preferably one or two, amino, guanidino, nitrogen containing heterocycle(s) or phenyl group(s) containing an amino, amidino or guanidino group;

R^{15} is hydrogen, lower alkyl optionally substituted with one or more, preferably one or two, amino, guanidino, nitrogen containing heterocycle(s) or phenyl group(s) containing an amino, amidino or guanidino group;

R^2 is hydrogen, hydroxysulfonyl, lower alkyl or lower alkenyl, wherein lower alkyl and lower alkenyl may be optionally substituted with acyl, carbamoyl, amino, mono-lower alkylamino or di-lower alkylamino;

R^3 is hydrogen, hydroxy, nitro, amino, acylamino, (lower alkylcarbamoyl)amino, carboxyl, lower alkoxy, lower alkoxycarbonyl, lower alkyl, lower alkenyl or lower alkynyl, wherein lower alkyl, lower alkenyl and lower alkynyl may be optionally substituted with hydroxy, amino, mono-lower alkylamino, di-lower alkylamino, lower alkoxycarbonyl or carbamoyl;

R^4 is alkyl, alkenyl, alkoxy or alkenyloxy which may be optionally substituted with lower alkyl, aryl, cycloalkyl or fluorine atom(s);

R^5 is $-CONH_2$, -CN or $-CH_2NH_2$;

X is a single bond, or an aryl, biphenyl or terphenyl group optionally containing one or more hetero atom(s) and/or being substituted with halogen atom(s) or lower alkyl;

Y is a single bond, $-CH_2-$, -CH(lower alkyl)-, -CONH- or -CON(lower alkyl)-;

Z is -O-, -NH- or -N(lower alkyl)-;

m is an integer of 0 to 4; and

n is an integer of 2 to 5;

with the proviso that when $-Y-(CH_2)_m-X-R^4$ is unsubstituted alkyl or aralkyl, then R^1 is not amino, R^2 and R^3 are not hydrogen, R^5 is not $-CONH_2$, and Z is not -O- or -NH- at the same time;

and pharmaceutically acceptable salts thereof.

The present invention also relates to a pharmaceutical composition comprising an Aerothricin of Formula (I) and a pharmaceutically acceptable carrier. Furthermore, the present invention relates to the use of such Aerothricins for the preparation of medicaments, as well as to processes and intermediates for the preparation of the Aerothricins of Formula (I). Additionally, the present invention relates to a method for the prophylactic and/or therapeutic treatment of infectious diseases caused by pathogenic microorganisms.

In this specification, the term "lower" is used to mean a group consisting of 1 to 6, preferably 1 to 4 carbon atom(s), unless otherwise indicated.

The term "alkyl" refers to a branched or straight chain monovalent saturated aliphatic hydrocarbon radical of one to twenty carbon atoms, preferably of one to sixteen carbon atoms. The term "lower alkyl" refers to a branched or straight chain monovalent alkyl radical of one to six carbon atoms, preferably one to four carbon atoms. This term is further exemplified by such radicals as methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, *i*-butyl, *tert*-butyl and the like.

The term "alkenyl" refers to an alkyl group containing one or more double bond(s) in the alkylene chain.

The term "alkynyl" refers to an alkyl group containing one or more triple bond(s) in the alkylene chain.

The term "alkoxy" refers to the group -O-R', where R' is an alkyl. The term "lower alkoxy" refers to the group -O-R', where R' is a lower alkyl.

The term "alkenyloxy" refers to an alkoxy group which contains one or more double bond(s) in the alkylene chain.

The term "acyl" refers to the group -C(O)-R', where R' is a lower alkyl. The term "acylamino" refers to an acyl group attached to an imino radical, i.e., -NH-.

The term "mono-lower alkylamino" refers to a lower alkyl group attached to an imino radical, i.e., -NH-. The term "di-lower alkylamino" refers to two independently selected lower alkyl groups attached to a nitrogen atom, i.e., -N(-lower alkyl)-lower alkyl. The term "tri-lower alkylammonio" means tri-lower alkylammonio containing three independently selected C_{1-3}-alkyl groups.

The term "lower alkoxycarbonyl" refers to the group -C(O)OR', where R' is a lower alkyl.

The term "(lower alkylcarbamoyl)amino" refers to the group -NHCONH-R', where R' is a lower alkyl.

The term "halogen atom" refers to fluorine, chlorine, bromine and iodine.

The term "aryl" refers to a monovalent carbocyclic aromatic radical (e.g. phenyl), or two condensed carbocyclic rings (e.g. naphtyl) optionally mono-, di- or tri-substituted, independently, with lower alkyl, trifluoromethyl, halogen and the like.

The term "nitrogen containing heterocycle" refers to a saturated, unsaturated or aromatic monovalent cyclic radical containing at least one nitrogen atom.

The term "heteroaryl" refers to an aromatic monovalent mono- or poly-carbocyclic radical containing at least one heteroatom, i.e. nitrogen, sulfur or oxygen. Examples of heteroaryl residues with one or more nitrogen atoms are pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, triazinyl and imidazolyl.

The term "cycloalkyl" refers to a monovalent carbocyclic radical of three to ten carbon atoms, preferably of three to six carbon atoms.

The term "pharmaceutically acceptable salts" embraces salts of the Aerothricis of the Formula (I) with inorganic or organic acid such as hydrochloric acid, hydrobromic acid, nitric acid, sulfuric acid, phosphoric acid, citric acid, formic acid, maleic acid, acetic acid, trifluoroacetic acid, succinic acid, tartaric acid, methanesulfonic acid, p-toluenesulfonic acid and the like, which are non-toxic to living organisms.

Each substituent of Formula (I) in the above is explained in more detail hereafter.

In the definition of R^1, the term "tri-lower alkylammonio" preferably means trimethylammonio and triethylammonio.

In the definition of R^{10} and R^{11}, the term "heteroaryl" preferably means 2-pyridyl, 2-pyrazinyl, 2-pyrimidinyl, 2-pyridazinyl, 2-triazinyl, 2-imidazolyl and the like, more preferably 2-pyridyl and 2-imidazolyl, most preferably 2-pyridyl. The term "lower alkyl" preferably means an alkyl chain consisting of 1 to 6 carbon atoms such as methyl, ethyl, *n*-propyl, *iso*-propyl, *n*-butyl, *iso*-butyl, *sec*-butyl, *n*-pentyl, neopentyl, *tert*-pentyl, and *n*-hexyl; preferably methyl, ethyl, *n*-propyl or *n*-butyl, most preferably methyl, ethyl or *n*-propyl. The term "nitrogen containing heterocycles" preferably means morpholino, piperazinyl, N-methylpiperazinyl, pyrrolidinyl, piperidinyl, imidazolidinyl, pyrazolidinyl, imidazolyl, pyrazolyl, triazolyl, pyridinyl, pyrazinyl and the like, more preferably piperazinyl and morpholino, most preferably piperazinyl. The term "phenyl group(s) containing an amino, amidino or guanidino group" preferably means 4-aminophenyl, 4-amidinophenyl, 4-guanidinophenyl and the like.

In the definition of R^{13}, the term "a residue derived from natural or unnatural amino acids" preferably means hydrogen or lower alkyl which may be substituted with hydroxy, amino, guanidino, methylthio, mercapto, carbamoyl, carboxy, phenyl, hydroxyphenyl, aminophenyl, imidazolyl or indolyl and the like. Preferable embodiment of R^{13} is lower alkyl substituted with amino or guanidino such as aminomethy, 2-aminoethyl, 3-aminopropyl, 4-aminobutyl, 4-guanidinobutyl.

In the definition of R^{14}, the term "lower alkyl" means the same as defined for R^{10} and R^{11}. Preferably, it means an alkyl chain consisting of 2 to 5 carbon atoms such as ethyl, propyl, butyl and pentyl. The term "nitrogen containing heterocycles" means the same as defined for R^{10} and R^{11}. Preferably, it means morpholino, piperazinyl, N-methylpiperazinyl, pyrrolidinyl, piperidinyl, imidazolidinyl, pyrazolidinyl, imidazolyl, pyrazolyl, triazolyl, pyridinyl, pyrazinyl and the like, more preferably piperazinyl and morpholino. The term "phenyl group(s) containing an amino, amidino or guanidino group" preferably means 4-aminophenyl, 4-amidinophenyl, 4-guanidinophenyl and the

like. Preferable embodiment of R^{14} is 2-aminoethyl, 3-aminopropyl, 4-aminobutyl, 2-guanidinoethyl, 3-guanidinopropyl, 2-piperazinoethyl, 2-morpholinoethyl, 4-aminophenethyl and the like.

In the definition of R^{15}, the terms "lower alkyl", "nitrogen containing heterocycles" and "phenyl group(s) containing an amino, amidino or guanidino group" are the same as defined for R^{14}. Preferable embodiment of R^{15} is 2-aminoethyl, 3-aminopropyl, 4-aminobutyl, 2-guanidinoethyl, 3-guanidinopropyl, 2-piperazinoethyl, 2-morpholinoethyl, 4-aminophenethyl and the like.

Preferable embodiments of $-N(R^{10})-R^{11}$ [wherein R^{10} and R^{11} are as defined above] are amino, 5-aminopyrid-2-ylamino, methylamino, ethylamino, propylamino, (2-aminoethyl)amino, (3-aminopropyl)amino, [3-[(3-aminopropyl)amino]propyl]amino, (2-piperazinylethyl)amino, (2-morpholinoethyl)amino, N,N-dimethylamino, N,N-diethylamino, N,N-dipropylamino, N,N-ethylmethylamino, N,N-bis(2-aminoethyl)amino, N,N-bis(3-aminopropyl)amino, N,N-bis(4-aminobutyl)amino, N,N-bis(2-piperazinylethyl)amino, N,N-bis(2-morpholinoethyl)amino, N,N-bis(2-guanidinoethyl)amino, N,N-bis(3-guanidinopropyl)amino, N,N-bis(2-pyridin-2-ylethyl)amino, N,N-bis(imidazol-2-ylmethyl)amino, N-(2-aminoethyl)-N-(3-aminopropyl)amino, N-(3-aminopropyl)-N-(2-piperazinylethyl)amino, N-(3-aminopropyl)-N-(2-pyridin-2-ylethyl)amino and the like. More preferable embodiments are amino, 5-aminopyrid-2-ylamino, N,N-dimethylamino, (2-aminoethyl)amino, (3-aminopropyl)amino, [3-[(3-aminopropyl)amino]propyl]amino, (2-piperazinylethyl)amino, N,N-bis(2-aminoethyl)amino, N,N-bis(3-aminopropyl)amino, N,N-bis(4-aminobutyl)amino, N,N-bis(2-piperazinylethyl)amino, N,N-bis(2-guanidinoethyl)amino, N,N-bis(3-guanidinopropyl)amino, N-(2-aminoethyl)-N-(3-aminopropyl)amino, N-(3-aminopropyl)-N-(2-piperazinylethyl)amino and the like. Most preferable embodiments are (3-aminopropyl)amino, N,N-bis(2-aminoethyl)amino, N,N-bis(3-aminopropyl)amino and N,N-bis(2-piperazinylethyl)amino.

In the definition of $-N(R^{15})-CO-CH[N(R^{10})R^{11}]-R^{13}$, the group $-CO-CH[N(R^{10})R^{11}]-R^{13}$ [wherein R^{10} and R^{11} are hydrogen; R^{13} is a residue derived from natural or unnatural amino acids] preferably means sarcosyl, glycyl, alanyl, ornitinyl, lysyl, valyl, leucyl, isoleucyl, tryptophyl, phenylalanyl, methionyl, seryl, tyrosyl, threonyl, cysteinyl, asparaginyl, glutaminyl, aspartyl, glutamyl, arginyl, histidyl, 2,3-diaminopropionyl, 2,4-diaminobutyryl, 2-amino-4-triazol-1-ylbutyryl and the like.

Preferable embodiments of $-N(R^{15})-CO-CH[N(R^{10})R^{11}]-R^{13}$ are acylamino groups derived from basic amino acids. Examples of such acylamino groups are ornitinylamino, lysylamino, arginylamino, histidylamino, 3-aminoprolylamino,

2,3-diaminopropionylamino, 2,4-diaminobutyrylamino, 2-amino-4-triazol-1-ylbutyrylamino, [3-amino-2-[bis(2-aminoethyl)amino]propionyl]amino, [4-amino-2-[bis(2-aminoethyl)amino]butyryl]amino, [5-amino-2-[bis(2-aminoethyl)amino]valeryl]amino, N-(3-aminopropyl)-N-(2,3-diaminopropionyl)amino, N-(3-aminopropyl)-N-(2,4-diaminobutyryl)amino, N-(3-aminopropyl)-N-(2,5-diaminovaleryl)amino, N-(3-aminopropyl)-N-(2,6-diaminohexanoyl)amino and the like; more preferably ornitinylamino, lysylamino, arginylamino, histidylamino, 2,3-diaminopropionylamino, 2,4-diaminobutyrylamino, [3-amino-2-[bis(2-aminoethyl)amino]propionyl]amino, [4-amino-2-[bis(2-aminoethyl)amino]butyryl]amino, [5-amino-2-[bis(2-aminoethyl)amino]valeryl]amino, N-(3-aminopropyl)-N-(2,3-diaminopropionyl)amino, N-(3-aminopropyl)-N-(2,4-diaminobutyryl)amino, N-(3-aminopropyl)-N-(2,5-diaminovaleryl)amino and N-(3-aminopropyl)-N-(2,6-diaminohexanoyl)amino, most preferably ornitinylamino, lysylamino, 2,4-diaminobutyrylamino, [4-amino-2-[bis(2-aminoethyl)amino]butyryl]amino, [5-amino-2-[bis(2-aminoethyl)amino]valeryl]amino, N-(3-aminopropyl)-N-(2,4-diaminobutyryl)amino, N-(3-aminopropyl)-N-(2,5-diaminovaleryl)amino and N-(3-aminopropyl)-N-(2,6-diaminohexanoyl)amino.

In the definition of R^1, preferable embodiment of

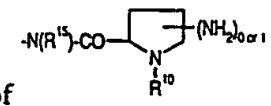
$$-N\begin{matrix}\nearrow (CH_2)_n\text{-}N(R^{15})\text{-}CO\text{-}CH[N(R^{10})R^{11}]\text{-}R^{13} \\ \searrow (CH_2)_n\text{-}N(R^{15})\text{-}CO\text{-}CH[N(R^{10})R^{11}]\text{-}R^{13},\end{matrix}$$

is bis[2-(ornitylamino)ethyl]amino, bis-[3-(ornitylamino)propyl]amino, [2-(lysylamino)ethyl]amino, bis-[3-(lysylamino)propyl]amino and the like.

In the definition of R^1, preferable embodiment of

$$-N\begin{matrix}\nearrow CO\text{-}CH[N(R^{10})R^{11}]\text{-}R^{13} \\ \searrow (CH_2)_n\text{-}N(R^{15})\text{-}CO\text{-}CH[N(R^{10})R^{11}]\text{-}R^{13}\end{matrix}$$

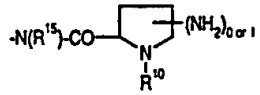
is N-ornityl-N-[2-(ornitylamino)ethyl]-amino, N-ornityl-N-[3-(ornitylamino)propyl]-amino, N-ornityl-N-[3-(lysylamino)propyl]amino, N-ornityl-N-[3-(lysylamino)propyl]-amino, N-lysyl-N-[2-(ornitylamino)ethyl]amino, N-lysyl-N-[3-(ornitylamino)propyl]-amino, N-lysyl-N-[2-(lysylamino)ethyl]amino, N-lysyl-N-[3-(lysylamino)propyl]amino and the like.

In the definition of R^1, preferable embodiment of  is prolylamino, 3-aminoprolylamino, 4-aminoprolylamino, N-(3-aminopropyl)-N-prolylamino, (2-aminoethyl)prolylamino and the like.

The term "-NHCOCH(R^{13})-NHCOCH(NH_2)-R^{13}" [wherein R^{13} is as defined above] preferably means ornityl-ornitylamino, lysyl-ornitylamino, ornityl-lysylamino, lysyl-lysylamino and the like.

In the term "-N(R^{15})-CO-R^{14}" [wherein R^{14} and R^{15} are as defined above], the term "nitrogen containing heterocycle" and the term "phenyl group(s) containing an amino, amidino or guanidino group" are as defined above.

Preferable embodiments of -N(R^{15})-CO-R^{14} are 3-aminopropionylamino, 3-guanidinopropionylamino, 3-piperazinylpropionylamino, (3-pyridin-3-ylpropionyl)amino, [3-(4-aminophenyl)propionyl]amino, N-(3-aminopropionyl)-N-(3-aminopropyl)amino and the like.

In a preferred aspect, R^1 is -N(R^{10})-R^{11}, wherein R^{10} and R^{11} are as defined above. In another preferred aspect, R^1 is -N(R^{15})-CO-CH[N(R^{10})R^{11}]-R^{13}, wherein R^{10}, R^{11}, R^{13} and R^{15} are as defined above. In another preferred aspect, R^1 is -N(R^{15})-CO-R^{14}, wherein R^{14} and R^{15} are as defined above. In another preferred aspect, R^1 is  , wherein R^{10} and R^{15} are as defined above. In another preferred aspect, R^1 is -NHCOCH(R^{13})-NHCOCH(NH_2)-R^{13}, wherein R^{13} is as defined above. In another preferred aspect, R^1 is tri-lower alkylammonio. In still another preferred aspect, R^1 is amino or guanidino.

In the definition of R^2, the term "lower alkyl optionally substituted with acyl, carboxy, carbamoyl, amino, mono-lower alkylamino or di-lower alkylamino" preferably means methyl, ethyl, *n*-propyl, isopropyl, butyl, oxo-lower alkyl, carboxy-lower alkyl, carbamoyl-lower alkyl, amino-lower alkyl and the like, more preferably methyl, ethyl, *n*-propyl, *n*-butyl, 2-oxopropyl, carboxymethyl, carbamoylmethyl, 3-aminopropyl and the like.

The term "lower alkenyl optionaly substituted with acyl, carboxy, carbamoyl, amino, mono-lower alkylamino or di-lower alkylamino" preferably means allyl, 2-butenyl, 3-butenyl and the like, more preferably allyl.

In a preferred aspect, R^2 is hydrogen, hydroxysulfonyl or lower alkyl such as methyl or ethyl.

In the definition of R^3, the term "acylamino" preferably means lower alkylcarbonylamino such as acetylamino, propionylamino or isobutyrylamino, or an acylamino group derived from natural or unnatural amino acids such as sarcosylamino, glycylamino, alanylamino, ornitylamino, lysylamino, prolylamino, valylamino, leucylamino, isoleucylamino, tryptophylamino, phenylalanylamino, methionylamino, serylamino, tyrosylamino, threonylamino, cysteinylamino, asparaginylamino, glutamylamino, aspartylamino, glutamylamino, arginylamino, histidylamino and the like; preferably sarcosylamino, glycylamino, alanylamino, lysylamino, prolylamino and the like.

The term "(lower-alkylcarbamoyl)amino" preferably means methylcarbamoylamino, ethylcarbamoylamino, propylcarbamoylamino, butylcarbamoylamino and the like, more preferably methylcarbamoylamino or ethylcarbamoylamino.

The term "lower alkoxy" preferably means methoxy, ethoxy, propoxy, butoxy and the like, more preferably methoxy and ethoxy.

The term "lower alkoxycarbonyl" preferably means methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl, butoxycarbonyl and the like, more preferably methoxycarbonyl and ethoxycarbonyl.

The term "lower alkyl which may be optionally substituted with hydroxy, amino, mono-lower alkylamino, di-lower alkylamino, lower alkoxycarbonyl or carbamoyl" preferably means methyl, ethyl, propyl, aminomethyl, aminoethyl, aminopropyl, hydroxymethyl, hydroxyethyl, methylaminomethyl, 2-(methylamino)ethyl, 3-(methylamino)propyl, dimethylaminomethyl, 2-(dimethylamino)ethyl, 3-(dimethylamino)propyl, 2-(methoxycarbonyl)ethyl, 2-(carbamoyl)ethyl and the like.

The term "lower alkenyl which may be optionally substituted with hydroxy, amino, mono-lower alkylamino, di-lower alkylamino, lower alkoxycarbonyl or carbamoyl" preferably means vinyl, 2-(methoxycarbonyl)vinyl, 2-(carbamoyl)vinyl and the like.

The term "lower alkynyl which may be optionally substituted with hydroxy, amino, mono-lower alkylamino, di-lower alkylamino, lower alkoxycarbonyl or carbamoyl"

preferably means ethynyl, propynyl, hydroxypropynyl, aminopropynyl, diethylaminopropynyl and the like.

In a preferred aspect, R^3 is hydrogen, hydroxy, nitro, amino or acylamino. In another preferred aspect R^3 is (lower alkylcarbamoyl)amino, carboxyl, lower alkoxy or lower alkoxycarbonyl.

In the definition of R^4, the term "alkyl, alkenyl, alkoxy or alkenyloxy" preferably means an alkyl, alkenyl, alkoxy or alkenyloxy group containing 3 to 16 carbon atoms, such as propyl, butyl, pentyl, hexyl, heptyl, octyl, oct-4-enyl, oct-6-enyl, nonanyl, decyl, undecyl, dodecyl, tridecyl, tetradecyl, pentadecyl, hexadecyl, propoxy, butoxy, pentyloxy, hexyloxy, heptyloxy, octyloxy, oct-4-enyloxy, oct-6-enyloxy, nonanyloxy, non-5-enyloxy, decyloxy and the like.

The term "lower alkyl" preferably means methyl, ethyl, propyl, butyl, pentyl, more preferably methyl or ethyl.

The term "aryl" means an aryl group which may optionally be substituted with lower alkyl, trifluoromethyl or halogen atom(s) such as phenyl, naphtyl, 3-fluorophenyl, 3-bromophenyl, 3-chlorophenyl, 4-fluorophenyl, 4-bromophenyl, 4-chlorophenyl, 3-methylphenyl, 4-methylphenyl, 4-trifluoromethylphenyl.

The term "cycloalkyl" preferably means cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, adamantyl and the like.

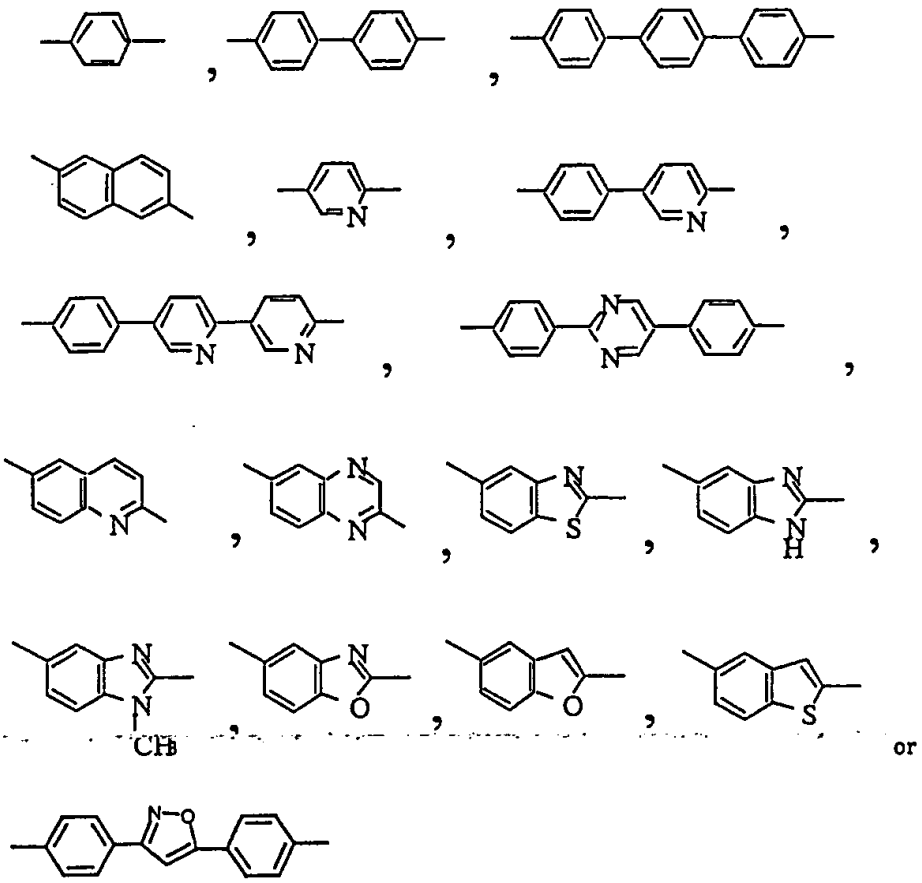
The term "alkyl, alkenyl, alkoxy or alkenyloxy which may be optionally substituted with lower alkyl, aryl, cycloalkyl or fluorine atom(s)" preferably means 5-methylhexyl, 1-methyltridecyl, 2-ethylbutoxy, 4-methylpentyloxy, 2-propylpentyloxy, 2-ethylhexyloxy, 3,7-dimethyloctyloxy, 2-phenylethoxy, 2-(4-fluorophenyl)ethoxy, 2-(4-chlorophenyl)ethoxy, 2-(3-fluorophenyl)ethoxy, 2-(4-trifluorophenyl)ethoxy, 3-phenylpropoxy, 2-naphtylethoxy, 3-naphtylpropoxy, 2-cyclopropylethoxy , 2-cyclobutylethoxy, 2-cyclopentylethoxy, 3-cyclopentylpropoxy, 2-cyclohexylethoxy, 3-cyclohexylpropoxy, 3,3-diphenylpropoxy, 3,3,3- trifluoropropoxy, 4,4,4-trifluorobutoxy, 5,5,5-trifluoropentyloxy and the like.

In a preferred aspect, R^4 is alkyl or alkoxy which may be optionally substituted with lower alkyl, aryl, cycloalkyl or fluorine atom(s).

Preferable embodiments of R^5 are $-CONH_2$ or $-CH_2NH_2$.

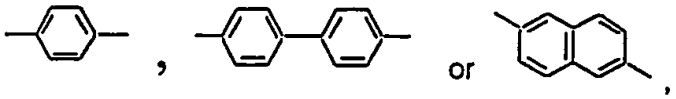
In the definition of X, the term "hetero atom" preferably means nitrogen, sulfur and oxygen.

The term "aryl, biphenyl or terphenyl optionally containing one or more hetero atom(s)" preferably means



and the like, which may be further substituted with halogen atom(s) or lower alkyl. The open-ended lines in the formulas above indicate the preferred linkage in the corresponding position.

The most preferable embodiment of X is a single bond,



which may be further substituted with halogen atom(s) or lower alkyl, preferably methyl.

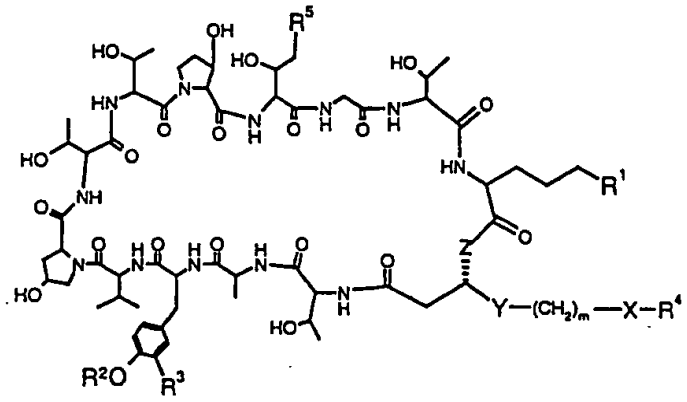
In the definition of Y, the term "lower alkyl" preferably means an alkyl group consisting of 1 to 3 carbon atoms, e.g. methyl, ethyl or propyl. The preferable embodiment of Y is a single bond, $-CH_2-$, $-CH(CH_3)-$, -CONH- or $-CON(CH_3)-$, more preferably a single bond, $-CH(CH_3)-$ or -CONH-.

In the definition of Z, the term "-N(lower alkyl)-" preferably means an N-alkyl group consisting of 1 to 3 carbon atoms, e.g. N-methyl, N-ethyl or N-propyl. A preferable embodiment of Z is -O- ; another preferable embodiment of Z is -NH-.

m is an integer of 0 to 4, preferably 0 to 2.

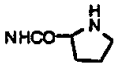
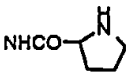
Preferred Aerothricins in accordance with the present invention are Aerothricins 2 and 4 to 131 as exemplified in the following Table 1.

Table 1

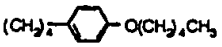
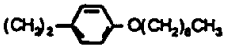
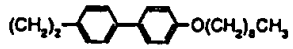
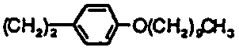
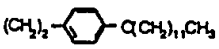
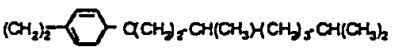
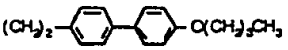
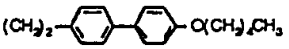
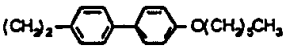
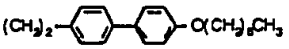
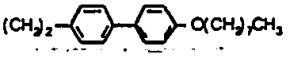
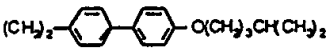
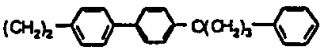
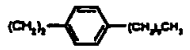
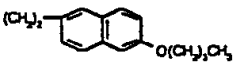
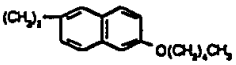
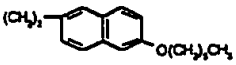


(I)

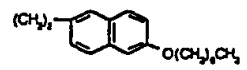
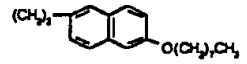
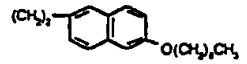
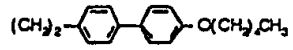
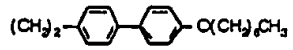
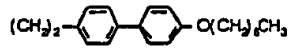
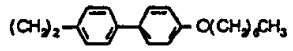
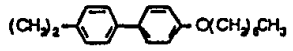
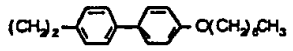
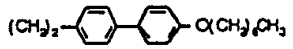
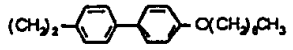
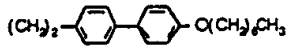
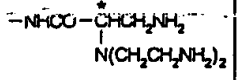
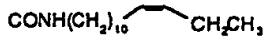
Formula (I)

Compound name	R^1	R^2	R^3	R^5	Z	$Y-(CH_2)_m-X-R^4$
Aerothricin 1 (starting material)	NH_2	H	H	$CONH_2$	O	$CH(CH_3)-(CH_2)_{11}CH_3$
Aerothricin 2	NH_2	H	OH	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 3 (starting material)	NH_2	H	H	$CONH_2$	O	$(CH_2)_{12}CH_3$)
Aerothricin 4	$NHC(=NH)NH_2$	H	H	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 5	NH_2	CH_3	H	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 6	NH_2	CH_2CH_3	H	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 7	NH_2	$CH_2-CH=CH_2$	H	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 8	NH_2	CH_2COCH_3	H	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 9	NH_2	CH_2CO_2H	H	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 10	NH_2	CH_2CONH_2	H	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 11	NH_2	CH_3	OCH_3	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 12	$N(CH_3)_2$	CH_3	H	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 13	$N(CH_3)_2$	H	H	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 14	$NHCOCH_2NHCH_3$	H	H	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 15		H	H	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 16	NH_2	H	NO_2	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 17	NH_2	H	NH_2	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 18	NH_2	H	$NHCOCH_2NH_2$	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 19	NH_2	H	$NHCOCH_3$	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 20	NH_2	H	$NHCOCH(CH_3)NH_2$	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 21	$NHCOCH_2NH_2$	H	$NHCOCH_2NH_2$	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 22	NH_2	H	$NHCONHCH_3$	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 23	NH_2	H	$NHCONHCH_2CH_3$	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 24	NH_2	H	$CH=CH-CO_2CH_3$	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 25	$N(CH_3)_2$	H	NO_2	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 26	$NHCOCH_2NHCH_3$	H	NO_2	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 27		H	NO_2	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 28	$NHCOCH_2NH_2$	H	NO_2	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 29	$NHCOCH_2NH_2$	H	NH_2	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 30	$N(CH_3)_2$	H	$NHCOCH(CH_3)N(CH_3)_2$	$CONH_2$	O	$(CH_2)_{12}CH_3$

Formula (I)

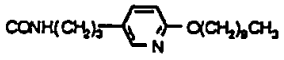
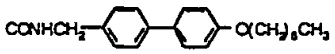
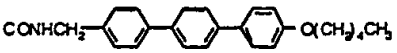
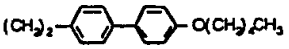
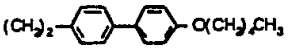
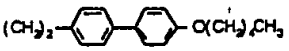
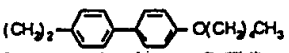
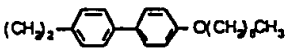
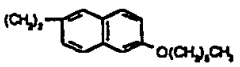
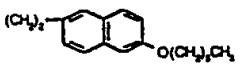
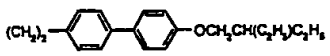
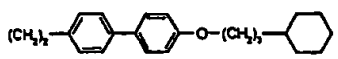
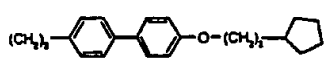
Compound name	R^1	R^2	R^3	R^5	Z	$Y-(CH_2)_m-X-R^4$
Aerothricin 31	NH_2	H	H	CH_2NH_2	O	$(CH_2)_{12}CH_3$
Aerothricin 32	NH_2	H	H	CN	O	$(CH_2)_{12}CH_3$
Aerothricin 33	NH_2	H	H	$CONH_2$	NH	
Aerothricin 34	NH_2	H	H	$CONH_2$	NH	
Aerothricin 35	NH_2	H	H	$CONH_2$	NH	
Aerothricin 36	NH_2	H	H	$CONH_2$	NH	
Aerothricin 37	NH_2	H	H	$CONH_2$	NH	
Aerothricin 38	NH_2	H	H	$CONH_2$	NH	
Aerothricin 39	NH_2	H	NO_2	$CONH_2$	NH	$(CH_2)_{12}CH_{13}$
Aerothricin 40	NH_2	H	H	$CONH_2$	NH	
Aerothricin 41	NH_2	H	H	$CONH_2$	NH	
Aerothricin 42	NH_2	H	H	$CONH_2$	NH	
Aerothricin 43	NH_2	H	H	$CONH_2$	NH	
Aerothricin 44	NH_2	H	H	$CONH_2$	NH	
Aerothricin 45	NH_2	H	H	$CONH_2$	NH	
Aerothricin 46	NH_2	H	H	$CONH_2$	NH	
Aerothricin 47	NH_2	H	H	$CONH_2$	NH	
Aerothricin 48	NH_2	H	H	$CONH_2$	NH	
Aerothricin 49	NH_2	H	H	$CONH_2$	NH	
Aerothricin 50	NH_2	H	H	$CONH_2$	NH	

Formula (I)

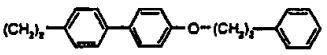
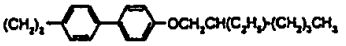
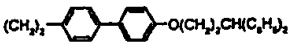
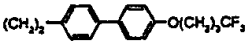
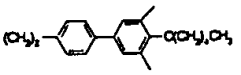
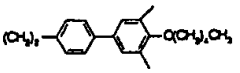
Compound name	R^1	R^2	R^3	R^5	Z	$Y-(CH_2)_m-X-R^4$
Aerothricin 51	NH_2	H	H	$CONH_2$	NH	
Aerothricin 52	NH_2	H	H	$CONH_2$	NH	
Aerothricin 53	NH_2	H	H	$CONH_2$	NH	
Aerothricin 54	NH_2	H	NO_2	$CONH_2$	NH	
Aerothricin 55	NH_2	H	NO_2	$CONH_2$	NH	
Aerothricin 56	NH_2	H	NH_2	$CONH_2$	NH	
Aerothricin 57	NH_2	H	$NHCOCH_3$	$CONH_2$	NH	
Aerothricin 58	NH_2	H	$NHCOCH(CH_3)NH_2$	$CONH_2$	NH	
Aerothricin 59	NH_2	H	$NHCOCH_2NH_2$	$CONH_2$	NH	
Aerothricin 60	NH_2	H	$NHCOCH_2NHCH_3$	$CONH_2$	NH	
Aerothricin 61	NH_2	H	$NHCO(CH_2)_2NH_2$	$CONH_2$	NH	
Aerothricin 62	NH_2	H	$NHCONHCH_2CH_3$	$CONH_2$	NH	
Aerothricin 63		H	H	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 64	NH_2	H	H	$CONH_2$	NH	$CONH(CH_2)_{10}CH_3$
Aerothricin 65	NH_2	H	H	$CONH_2$	NH	$CONH(CH_2)_{12}CH_3$
Aerothricin 66	NH_2	H	H	$CONH_2$	NH	$CONH(CH_2)_{14}CH_3$
Aerothricin 67	NH_2	H	H	$CONH_2$	NH	
Aerothricin 68	NH_2	H	H	$CONH_2$	NH	
Aerothricin 69	NH_2	H	H	$CONH_2$	NH	$CON(CH_3)-(CH_2)_{12}CH_3$

* (S) configuration

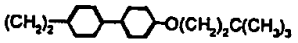
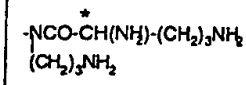
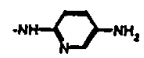
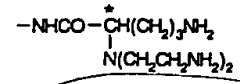
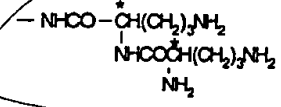
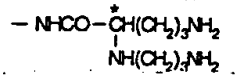
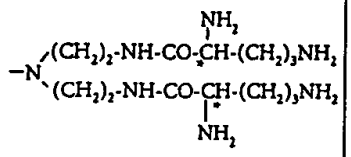
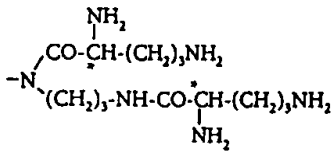
Formula (I)

Compound name	R^1	R^2	R^3	R^5	Z	$Y-(CH_2)_m-X-R^4$
Aerothricin 70	NH_2	H	H	$CONH_2$	NH	$CON(CH_3)-(CH_2)_{14}CH_3$
Aerothricin 71	NH_2	H	H	$CONH_2$	NH	
Aerothricin 72	NH_2	H	H	$CONH_2$	NH	
Aerothricin 73	NH_2	H	H	$CONH_2$	NH	
Aerothricin 74	$NHC(=NH)NH_2$	H	H	$CONH_2$	NH	$CONH(CH_2)_{14}CH_3$
Aerothricin 75	$N(CH_3)2$	H	H	$CONH_2$	NH	$CONH(CH_2)_{14}CH_3$
Aerothricin 76	NH_2	CH_3	H	$CONH_2$	NH	$CONH(CH_2)_{14}CH_3$
Aerothricin 77	NH_2	H	NO_2	$CONH_2$	NH	$CONH(CH_2)_{14}CH_3$
Aerothricin 78	NH_2	H	NH_2	$CONH_2$	NH	$CONH(CH_2)_{14}CH_3$
Aerothricin 79	NH_2	H	$NHCONHCH_2CH_3$	$CONH_2$	NH	$CONH(CH_2)_{14}CH_3$
Aerothricin 80	NH_2	H	$NHCOCH_3$	$CONH_2$	NH	$CONH(CH_2)_{14}CH_3$
Aerothricin 81	NH_2	H	$NHCOCH_2NH_2$	$CONH_2$	NH	$CONH(CH_2)_{14}CH_3$
Aerothricin 82	$N(CH_3)2$	H	H	$CONH_2$	NH	
Aerothricin 83	$N(CH_3)2$	CH_3	H	$CONH_2$	NH	
Aerothricin 84	NH_2	CH_3	H	$CONH_2$	NH	
Aerothricin 85	NH_2	CH_3	NO_2	$CONH_2$	NH	
Aerothricin 86	NH_2	CH_3	NO_2	$CONH_2$	NH	
Aerothricin 87	NH_2	CH_3	H	$CONH_2$	NH	
Aerothricin 88	$N(CH_3)2$	H	H	$CONH_2$	NH	
Aerothricin 89	NH_2	H	H	$CONH_2$	NH	
Aerothricin 90	NH_2	H	H	$CONH_2$	NH	
Aerothricin 91	NH_2	H	H	$CONH_2$	NH	
Aerothricin 92	NH_2	H	H	$CONH_2$	NH	

Formula (I)

Compound name	R^1	R^2	R^3	R^5	Z	$Y-(CH_2)_m-X-R^4$
Aerothricin 93	NH_2	H	H	$CONH_2$	NH	
Aerothricin 94	NH_2	H	H	$CONH_2$	NH	
Aerothricin 95	NH_2	H	H	$CONH_2$	NCH_3	$(CH_2)_{14}CH_3$
Aerothricin 96	NH_2	H	CO_2H	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 97	NH_2	H	H	$CONH_2$	NH	
Aerothricin 98	NH_2	H	H	$CONH_2$	NH	
Aerothricin 99	NH_2	H	H	$CONH_2$	NH	
Aerothricin 100	bis(2-aminoethyl)-amino	H	H	$CONH_2$	NH	
Aerothricin 101	L-ornitinylamino	H	H	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 102	L-lysylamino	H	H	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 103	L-argininylamino	H	H	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 104	(2S)-(2,4-diamino-butyryl)amino	H	H	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 105	(2S)-(2,3-diamino-propionyl)amino	H	H	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 106	D-ornitinylamino	H	H	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 107	D-lysylamino	H	H	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 108	D-argininylamino	H	H	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 109	(2R)-(2,4-diamino-butyryl)amino	H	H	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 110	(2R)-(2,3-diamino-propionyl)amino	H	H	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 111	bis(2-aminoethyl)-amino	H	H	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 112	bis(3-aminopropyl)-amino	H	H	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 113	(3-aminopropyl)-amino	H	H	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 114	bis(2-piperazinyl-ethyl)amino	H	H	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 115	[N-(2-aminoethyl)-N-(3-aminopropyl)]amino	H	H	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 116	bis(2-guanidinyl-ethyl)amino	H	H	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 117	(2-piperazinyl-ethyl)amino	H	H	$CONH_2$	O	$(CH_2)_{12}CH_3$

Formula (I)

Compound name	R^1	R^2	R^3	R^5	Z	$Y-(CH_2)_m-X-R^4$
Aerothricin 118	(2S)-(2-amino-4-triazol-1-ylbutyryl)amino	H	H	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 119	L-histidylamino	H	H	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 120	(2-cyanoethyl)-amino	H	H	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 121	trimethyl-ammonio (iodide)	H	H	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 122	NH_2	SO_3H	H	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 123	NH_2	H	H	$CONH_2$	NH	
Aerothricin 124		H	H	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 125		H	H	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 126	$-NHCH_2CH-(CH_2NH_2)_2$	H	H	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 127		H	H	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 128		H	H	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 129		H	H	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 130		H	H	$CONH_2$	O	$(CH_2)_{12}CH_3$
Aerothricin 131		H	H	$CONH_2$	O	$(CH_2)_{12}CH_3$

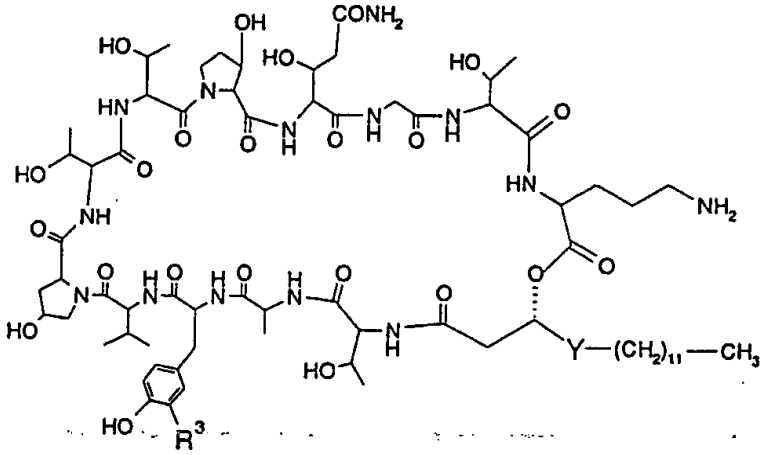
* (R) configuration

Particularly preferred are the Aerothricins selected from the group consisting of Aerothricins 2, 4 to 32, 63, 96- 99, 101 to 131. Also particularly preferred are the Aerothricins selected from the group consisting of Aerothricins 14, 15, 21, 26-29, 63, 98, 99, 101-131.

Aerothricins represented by Formula (I) can be produced according to the following methods.

Process A

Aerothricins of the Formula (II) can be produced by cultivating a microorganism belonging to *Deuteromycotina* capable of producing Aerothricins 1, 2 and 3 [Aerothricin 3 (= WF11243) is described in Reference Example 1] under aerobic conditions in an aqueous or a solid medium and isolating Aerothricins 1, 2 and 3 from the culture.

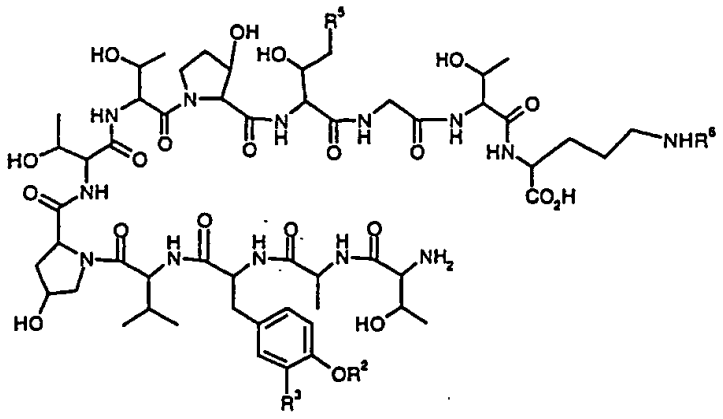


(II)

[wherein R^3 is hydrogen or hydroxy, Y is $-CH(CH_3)-$ or $-CH_2-$]

Process B

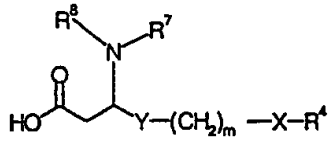
Aerothricins of the Formula (I) [wherein R^1 is amino; Y is -CONH-, -CON(lower alkyl)-, $-CH_2-$ or a single bond; Z is -NH- or -N(lower alkyl)-; R^2, R^3, R^4, R^5, X and m are as defined above] can be prepared by condensation of a compound of the Formula (III),



(III)

[wherein R^6 is an amino protecting group; R^2, R^3 and R^5 are as defined above],

with a compound of the Formula (IV),



(IV)

[wherein R^7 is an amino protecting group; R^8 is hydrogen or lower alkyl; R^4, X, Y and m are as defined above],

using a carboxy activating agent for peptide synthesis, followed by selective removal of the amino protecting group R^7 of the resulting linear peptide, the successive cyclization with a carboxy activating agent for peptide synthesis, and removal of the amino protecting group R^6.

Process C

Aerothricins of the Formula (I) [wherein R^3 is a nitro group; R^1, R^2, R^4, R^5, X, Y, Z and m are as defined above] can be prepared by nitration of Aerothricins of the Formula (I) [wherein R^3 is hydrogen; R^1, R^2, R^4, R^5, X, Y, Z and m are as defined above].

Process D

Aerothricins of the Formula (I) [wherein R^3 is an amino group; R^1, R^2, R^4, R^5, X, Y, Z and m are as defined above] can be prepared by reduction of the nitro group of Aerothricins of the Formula (I) [wherein R^3 is a nitro group; R^1, R^2, R^4, R^5, X, Y, Z and m are as defined above].

Process E

Aerothricins of the Formula (I) [wherein R^3 is acylamino or (lower alkylcarbamoyl)amino; R^1, R^2, R^4, R^5, X, Y, Z and m are as defined above] can be prepared by acylation of the amino group of Aerothricins of the Formula (I) [wherein R^3 is an amino group; R^1, R^2, R^4, R^5, X, Y, Z and m are as defined above] with acid chloride, acid anhydride, carboxylic acid/condensation agent or lower alkylcarbamoyl chloride, followed, if necessary, by removal of the amino protecting group.

Process F

Aerothricins of the Formula (I) [wherein R^1 is (3-aminopropyl)amino, (2-cyanoethyl)amino, 3-amino-2-(aminomethy)propyl]amino or $-N(R^{15})-COCH[NH(CH_2)_3NH_2]-R^{13}$ [wherein R^{13} and R^{15} are as defined above] can be prepared by reacting the amino group of Aerothricins of Formula (I) [wherein R^1 is an amino group or $-N(R^{15})-COCH(NH_2)-R^{13}$ [wherein R^{13} and R^{15} are as defined above]; R^2, R^3, R^4, R^5, X, Y, Z and m are as defined above] with acrylonitrile, ethoxymethylenemalononitrile or (1-ethoxyethylidene)malononitrile, followed by reduction of the resulting nitrile group(s) into amino group(s), and if necessary by removal of protecting group(s).

Process G

Aerothricins of the Formula (I) [wherein R^1 is $-N(R^{10})-R^{11}$ [wherein R^{10} and R^{11} are each independently selected from hydrogen, lower alkyl optionally substituted with one or more amino, guanidino, nitrogen containing heterocycle(s) or phenyl group(s) containing an amino, amidino or guanidino group] or $-N(R^{15})-CO-CH[N(R^{10})R^{11}]-R^{13}$ [wherein R^{10} and R^{11} are each a lower alkyl optionally substituted with one or more amino, amino-lower alkyl, guanidino, nitrogen containing heterocycle(s) or phenyl group(s) containing an

amino, amidino or guanidino group; R^{13} and R^{15} are as defined above]; R^2, R^3, R^4, R^5, X, Y, Z and m are as defined above] can be prepared by reductive alkylation of the amino group of Aerothricins of the Formula (I) [wherein R^1 is amino, (2-cyanoethyl)amino or -N(R^{15})-CO-CH[N(R^{10})R^{11}]-R^{13} [wherein R^{10} and R^{11} are each independently a hydrogen atom or (2-cyanoethyl)amino; R^{13} and R^{15} are as defined above]; R^2, R^3, R^4, R^5, X, Y, Z and m are as defined above] with an aldehyde of the Formula (V),

$$R^9\text{-CHO} \qquad (V)$$

[wherein R^9 is hydrogen, lower alkyl which may be further substituted with one or more protected amino, nitrogen containing heterocycle(s) or phenyl group(s) containing a protected amino group],

followed, if necessary, by removal of amino protecting group(s) or reduction of a cyano group.

Process H

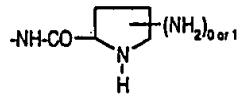
Aerothricins of the Formula (I) [wherein R^1 is -N(R^{10})-R^{11} [wherein R^{10} and R^{11} are each independently selected from hydrogen or heteroaryl substituted with one or two amino group(s)]; R^2, R^3, R^4, R^5, X, Y, Z and m are as defined above] can be prepared by reacting the amino group of Aerothricins of the Formula (I) [wherein R^1 is an amino group; R^2, R^3, R^4, R^5, X, Y, Z and m are as defined above] with a compound of the Formula (VI),

$$R^{12}\text{-Q} \qquad (VI)$$

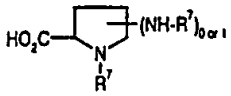
[wherein R^{12} is a nitrogen containing heteroaryl which may be further substituted with a protected amino or nitro group, Q is a halogen atom such as chloro or bromo],

followed, if necessary, by removal of an amino protecting group or reduction of a nitro group.

Process I-1

Aerothricins of the Formula (I) [wherein R^1 is  , -NHCO-$CH(NH_2)$-R^{13} [wherein R^{13} is a residue derived from natural or unnatural amino acids] or -NHCO-R^{14} [wherein R^{14} is as defined above]; R^2, R^3, R^4, R^5, X, Y, Z and m are as defined above] can be prepared by acylation of the amino group of Aerothricins of the Formula (I) [wherein R^1 is an amino group; R^2, R^3, R^4, R^5, X, Y, Z and m are as defined above] with an acid of the Formula (VII) or (VII'),

$$HO(O=)C\text{-}CH(NH\text{-}R^7)\text{-}R^{13} \qquad (VII)$$

 (VII')

[wherein R^{13} is a residue derived from natural or unnatural amino acids whose functional group is suitably protected, R^7 is an amino protecting group],

or an acid of the Formula (VIII),

$$HO(O=)C\text{-}R^{14} \qquad (VIII)$$

[wherein R^{14} is lower alkyl having one or more protected amino group(s), nitrogen containing heterocycle(s) or phenyl group(s) containing protected amino group];

followed, if necessary, by removal of the protecting group(s).

Process I-2

Aerothricins of the Formula (I) wherein R^1 is

$$-N\begin{matrix}\nearrow (CH_2)_n\text{-}N(R^{15})\text{-}CO\text{-}CH[N(R^{10})R^{11}]\text{-}R^{13} \\ \searrow (CH_2)_n\text{-}N(R^{15})\text{-}CO\text{-}CH[N(R^{10})R^{11}]\text{-}R^{13}\end{matrix}$$

[wherein R^{10}, R^{11}, R^{13}, R^{15}, and m are as defined above], or

$$-N\begin{matrix}\nearrow CO\text{-}CH[N(R^{10})R^{11}]\text{-}R^{13} \\ \searrow (CH_2)_n\text{-}N(R^{15})\text{-}CO\text{-}CH[N(R^{10})R^{11}]\text{-}R^{13}\end{matrix}$$

[wherein R^{10}, R^{11}, R^{13}, R^{15}, and m are as defined above]

can be prepared by acylation of the amino group of Aerothricins of the Formula (I), wherein R^1 is $-N(R^{10})-R^{11}$ [wherein R^{10} and R^{11} are both lower alkyl substituted with an amino group] or $-N(R^{15})-CO-CH[N(R^{10})R^{11}]-R^{13}$ [wherein R^{15} is lower alkyl substituted with an amino group; R^{10}, R^{11}, and R^{13} are as defined in Claim 1 with the proviso that the amino group(s) present in R^{10}, R^{11} and R^{13} are protected], with an acid of the Formula (VII)

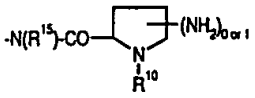
$$HO(O=)C-CH(NH-R^7)-R^{13} \qquad (VII)$$

[wherein R^{13} is a residue derived from natural or unnatural amino acids whose functional group is suitably protected, R^7 is an amino protecting group];

followed by removal of the protecting group(s).

Process I

Aerothricins of the Formula (I) [wherein R^1 is $-N(R^{15})-CO-CH[N(R^{10})R^{11}]-R^{13}$ [wherein R^{10} and R^{11} are hydrogen, R^{13} is as defined above and R^{15} is lower alkyl optionally substituted with one or more amino, guanidino, nitrogen containing heterocycle(s) or phenyl group(s) containing an amino, amidino or guanidino group],



[wherein R^{10} is hydrogen and R^{15} is lower alkyl optionally substituted with one or more amino, guanidino, nitrogen containing heterocycle(s) or phenyl group(s) containing an amino, amidino or guanidino group], or $-N(R^{15})-CO-R^{14}$ [wherein R^{15} is lower alkyl optionally substituted with one or more amino, guanidino, nitrogen containing heterocycle(s) or phenyl group(s) containing an amino, amidino or guanidino group, R^{14} is as defined above]; R^2, R^3, R^4, R^5, X, Y, Z and m are as defined above] can be prepared by mono N-alkylation of the amino group of Aerothricins of the Formula (I) [wherein R^1 is an amino group; R^2, R^3, R^4, R^5, X, Y, Z and m are as defined above] as described in process F, followed by acylation with a corresponding compound of the Formula (VII), (VII') or (VIII) as described in the process I, followed, if necessary, by removal of the protecting group(s).

Process K

Aerothricins of the Formula (I) [wherein R^1 is a guanidino group, $-N(R^{10})-R^{11}$ [wherein R^{10} and R^{11} are each independently selected from lower alkyl substituted with

guanidino or phenyl group(s) containing a guanidino group], -N(R^{15})-CO-CH[N(R^{10})R^{11}]-R^{13} [wherein R^{10}, R^{11} and R^{13} are as defined above and R^{15} is lower alkyl optionally substituted with one or more guanidino group(s), nitrogen containing heterocycle(s) or phenyl group(s) containing a guanidino group] or -N(R^{15})CO-R^{14} [wherein R^{14} is lower alkyl substituted with one or more guanidino group(s), nitrogen containing heterocycle(s) or phenyl group(s) containing a guanidino group; R^2, R^3, R^4, R^5, X, Y, Z and m are as defined above] can be prepared by reacting Aerothricins of the Formula (I) [wherein R^1 is an amino group; -N(R^{10})-R^{11} [wherein R^{10} and R^{11} are each independently selected from lower alkyl substituted with amino group(s) or phenyl group(s) containing an amino group], -N(R^{15})-CO-CH[N(R^{10})R^{11}]-R^{13} [wherein R^{10}, R^{11} and R^{13} are as defined above and R^{15} is lower alkyl optionally substituted with one or more amino group(s), nitrogen containing heterocycle(s) or phenyl group(s) containing an amino group]; or -NHCO-R^{14} [wherein R^{14} is lower alkyl substituted with one or more amino group(s), nitrogen containing heterocycle(s) or phenyl group(s) containing an amino group; R^2, R^3, R^4, R^5, X, Y, Z and m are as defined above] with an activated amidine derivative.

Process L

Aerothricins of the Formula (I) [wherein R^2 is lower alkyl or lower alkenyl optionally substituted with acyl, carboxy, carbamoyl, hydroxy, amino, mono-lower alkylamino or di-lower alkylamino; R^1, R^3, R^4, R^5, X, Y, Z and m are as defined above] can be prepared by O-alkylation of the phenolic hydroxyl group of Aerothricins of the Formula (I) [wherein R^2 is hydrogen; R^1, R^3, R^4, R^5, X, Y, Z and m are as defined above] with an alkylating agent.

Process M

Aerothricins of the Formula (I) [wherein R^3 is carboxyl, lower alkoxycarbonyl, lower alkyl, alkenyl or alkynyl which may be optionally substituted with hydroxy, amino, mono-lower alkylamino, di-lower alkylamino, lower alkoxycarbonyl or carbamoyl; R^2 is hydrogen; R^1, R^4, R^5, X, Y, Z and m are as defined above] can be prepared by iodination of Aerothricins of the Formula (I) [wherein R^2 and R^3 are hydrogen; R^1, R^4, R^5, X, Y, Z and m are as defined above] with an iodination agent, followed by palladium(0) catalyzed coupling of the resulting iodo derivative of the Formula (I) [wherein R^3 is an iodo; R^1, R^2, R^4, R^5, X, Y, Z and m are as defined above] with carbon monoxide, methyl acrylate and the like, and if necessary, by removal of the protecting group(s).

Process N

Aerothricins of the Formula (I) [wherein R^5 is -CN; R^1, R^2, R^3, R^4, X, Y, Z and m are as defined above] can be prepared by dehydration of the carbamoyl group of Aerothricins of the Formula (I) [wherein R^5 is $-CONH_2$; R^1, R^2, R^3, R^4, X, Y, Z and m are as defined above] with a dehydrating agent, and if necessary, by removal of the amino protecting group(s).

Process O

Aerothricins of the Formula (I) [wherein R^5 is $-CH_2NH_2$; R^1, R^2, R^3, R^4, X, Y, Z and m are as defined above] can be prepared by reduction of the carbamoyl or cyano group of Aerothricins of the Formula (I) [wherein R^5 is $-CONH_2$ or -CN; R^1, R^2, R^3, R^4, X, Y, Z and m are as defined above] with a reducing agent, and if necessary, by removal of the amino protecting group(s).

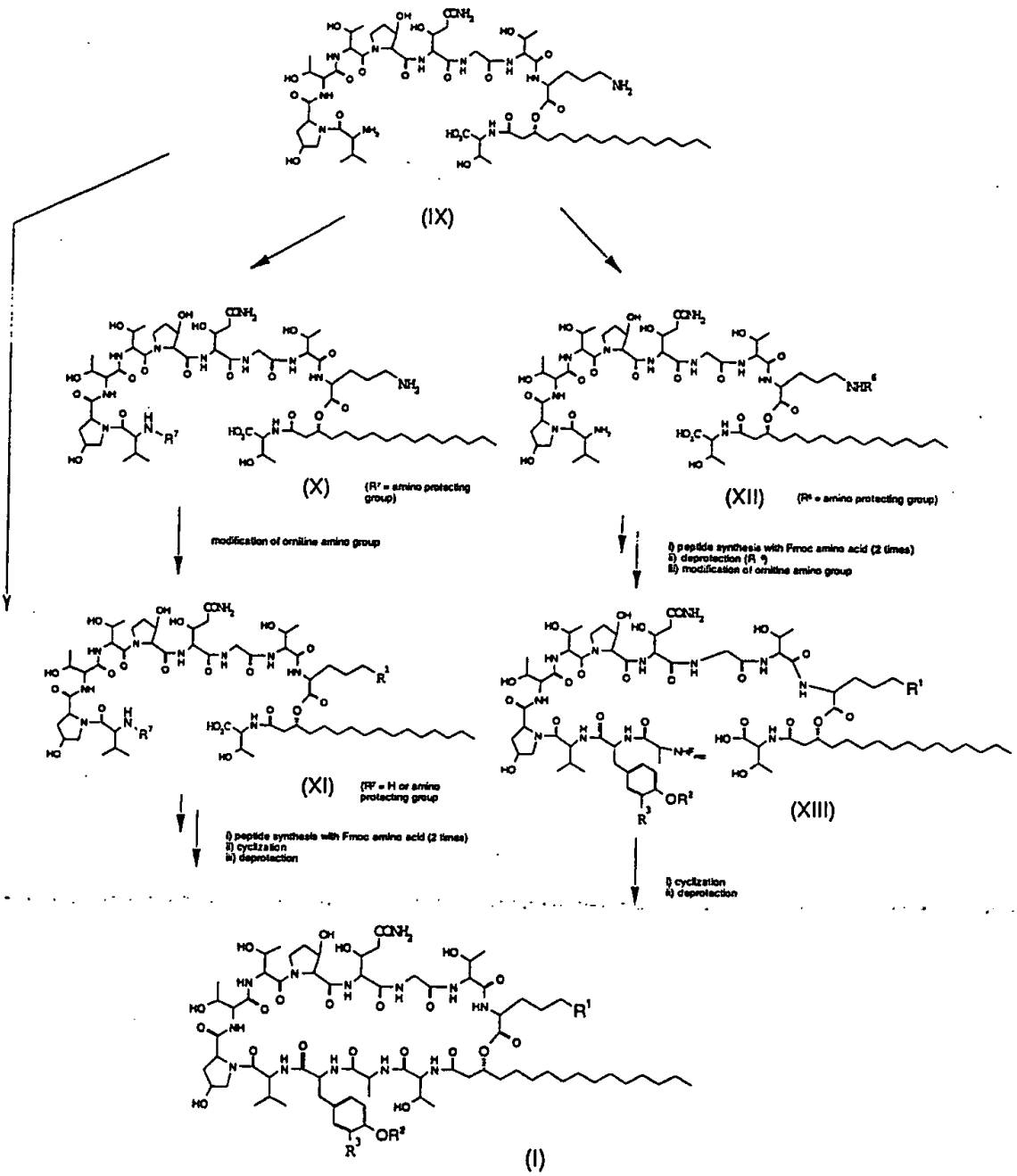
Process P

Aerothricins of the Formula (I) [wherein R^2 is hydroxysufonyl; R^1, R^3, R^4, R^5, X, Y, Z and m are as defined above] can be prepared by hydroxysulfonation of the tyrosine residue of Aerothricins of the Formula (I) [wherein R^2 is hydrogen; R^1, R^3, R^4, R^5, X, Y, Z and m are as defined above], followed by removal of protecting group(s).

Process Q

Aerothricins of the Formula (I) [wherein -Y-(CH_2)m-X-R^4 is *n*-tridecanyl or 1-methytridecanyl, R^5 is $-CONH_2$, Z is an oxygen atom and R^1, R^2, and R^3 are as defined above] can be prepared from the linear peptide of the Formula (IX) by the method outlined in Scheme 1.

The compound of above formula (III), wherein R^2, R^3 and R^5 are as defined above and R^6 is an amino protecting group, with the proviso that when R^5 is $-CONH_2$, then R^2 or R^3 are other than hydrogen, and salts thereof are new and are also subject of the present invention. Furthermore, the linear peptides of Formulas (IX), (X) and (XII) shown in Scheme 1 and optionally salts thereof are new and are also subject of the present invention.


(IX)
(X)
(R⁷ = amino protecting group)
(XII)
(R⁶ = amino protecting group)
modification of ornitine amino group
i) peptide synthesis with Fmoc amino acid (2 times)
ii) deprotection (R⁶)
iii) modification of ornitine amino group
(XI)
(R⁷ = H or amino protecting group
i) peptide synthesis with Fmoc amino acid (2 times)
ii) cyclization
iii) deprotection
(XIII)
i) cyclization
ii) deprotection
(I)

scheme 1

The Processes A to Q can be illustrated in more detail as follows:

Process A

The microorganism used in the present invention can be any strains including mutants and variants belonging to *Deuteromycotina* capable of producing Aerothricins 1, 2 and 3. Especially preferred is strain NR 7379 which was isolated from fallen leaves collected at Kagoshima pref. in Japan, and identified as a strain belonging to *Deuteromycotina*.

The cultural and morphological characteristics of strain NR 7379 are as follows:

1. Cultural characteristics

Corn meal agar (CMA): Growth was not extensive. The colonies reached 11 mm in diameter from inoculum (4.5 mm diam. agar plug) after 14 days at 25°C. They were plane and pale cream yellow. The reverse side was pale cream yellow. Colorless and mucilaginous exudates were present.

Miura's medium (LCA): Growth was not extensive. The colonies reached 11 mm in diameter from inoculum after 14 days at 25°C. They were plane and pale cream yellow. The reverse side was pale cream yellow. Exudates were absent.

Malt extract agar (MEA): Growth was not extensive. The colonies were pustuliform and attained a diameter of 18 mm from inoculum after 14 days at 25°C. The color of colonies was light yellowish brown. The reverse side was of the same color. Exudates were colorless and mucilaginous.

Potato-dextrose agar (PDA): Growth was not extensive. The colonies were pustuliform and reached 14 mm in diameter from inoculum after 14 days at 25°C. The color and texture of colonies were similar to those on MEA. Exudates were colorless and mucilaginous.

Germination was observed between 5°C and 30°C on CMA, LCA, MEA, and PDA.

2. Morphological characteristics

Mycelia were partly immersed, partly superficial, branched, septate, and pale brown to cream yellow. Conidiophores were formed from immersed mycelium. They were hyaline, septate, branched, irregular. Conidiogenous cells were on distinct conidiophores or irregular hyphae. They were enteroblastic, phialidic, terminal or subterminal. Terminal or subterminal phialides were variable in length and shape. They were cylindrical to lageniform and their length and width were up to 5.5 to 10 µm and 2.5 to 5.5 µm respectively. Irregularly filiform Conidiophores with lateral conidiogenous cells immediately below septa were often formed. Conidia were one-celled, hyaline, smooth, globose to subglobose, 2.0 to 5.5 µm in length and 2.0 to 5.0 µm in width.

On the basis of these distinct cultural and morphological characteristics, the present strain belonged to *Deuteromycotina* designated as Deuteromycotina NR 7379.

The strain denoted as Deuteromycotina NR 7379 has been deposited with the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, Japan in the name of Nippon Roche K.K., of 6-1, Shiba 2-chome, Minato-ku Tokyo, 105 Japan on June 16, 1998 under the Budapest Treaty as follows: Deuteromycotina NR 7379 (FERM BP-6391).

The cultivation in accordance with the process provided by the present invention can be carried out in a culture medium which contains customary nutrients usable by the microorganism being cultivated. As carbon sources there can be mentioned, for example, glucose, sucrose, starch, glycerol, molasses, dextrin and mixtures thereof. Nitrogen sources are, for example, soybean meal, cottonseed meal, meat extract, peptone, dried yeast, yeast extract, corn steep liquor, ammonium sulfate, sodium nitrate and mixtures thereof. Moreover, there may be added to the culture medium other organic or inorganic substances for promoting the growth of the microorganism and for increasing the production of Aerothricin 1. Examples of such substances are inorganic salts, such as calcium carbonate, sodium chloride, phosphates and the like.

The cultivation is carried out under aerobic conditions preferably in a liquid medium by submerged fermentation, or in a solid medium by static fermentation. A temperature of 20°C to 30°C, with an optimal temperature of 27°C is suitable for cultivation. The cultivation is preferably carried out at a pH of 3 to 9. The cultivation time depends on the conditions under which the cultivation is carried out. In general, it is sufficient to carry out the cultivation for 20 to 360 h.

For harvesting the objective Aerothricins 1, 2 and 3 from the cultures, separation methods which are usually employed to isolate metabolites produced by microbes from their cultures can be properly used. For example, Aerothricin 1, which is a methanol extractable amphoteric substance, is recovered advantageously by the following procedures.

That is, the whole culture solid obtained by solid state fermentation is extracted with an appropriate solvent to recover the proposed product. The solvents which can be used to extract the objective compound from the whole cultured solid include water-soluble organic solvents or hydrous solutions of water-soluble organic solvents, such as methanol, ethanol and hydrous alcohols.

For removing salts, water soluble substances, etc. from the resulting extract, use is made of, with advantage, solvent partition between water and water-immiscible organic solvents, such as *n*-butanol, ethyl acetate, etc. For removing coloring substances, fat-soluble substance or the like from the extract, use is made of, with advantage, solvent purification by methanol, ethanol, a mixture of acetonitrile-0.1% aqueous trifluoroacetic acid, etc.

For complete purification of Aerothricins, column chromatography is used with advantage. Carriers which can be used in such a column chromatography are such as YMC-GEL ODS (Yamamura Chemical Laboratories, Japan) or Preparative C18 (Waters Millipore Corporation). As an eluent, use is made of a solvent system consisting a mixture of aqueous trifluoroacetic acid and appropriate water-soluble organic solvents such as methanol, ethanol, acetonitrile, etc. The eluate fraction thus purified, which contains each component, can be subjected to concentration or freeze-drying to pulverize Aerothricins 1, 2 and 3.

Aerothricins 1, 2 and 3 were isolated as a trifluoroacetic acid salt, but the free Aerothricins 1, 2 and 3 can be prepared by the following procedure. Namely, Aerothricins 1, 2 and 3 trifluoroacetic acid salt are dissolved in water, to which was added one equivalent of sodium hydroxide, and the mixture is subjected to Sephadex LH-20 column Chromatography, followed by elution with a hydrous alcohol such as methanol-water, etc. to thereby obtain Aerothricins 1, 2 and 3 (free form), respectively.

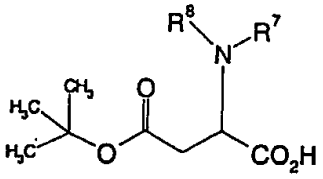
Process B

The starting compound of the Formula (III) can be prepared from Aerothricins of the Formula (I) [which includes Aerothricins 1 to 3 as well as those converted from

Aerothricins 1 to 3 by use of a process selected from the processes C to Q] by the method similar to that described in WO 96/30399. This method comprises alkaline hydrolysis of the lactone ring followed by enzymatic cleavage of the fatty acid chain. The preferable amino protecting groups for R^6 in the Formula (III) and R^8 in the Formula (IV) are *tert*-butoxycarbony (Boc) and 9-fluorenylmethyloxycarbonyl (Fmoc), respectively.

The starting compound of the Formula (III) can also be prepared from the linear peptide of the Formula (IX), obtained by fermentation of *Deuteromycotina*, by conventional peptide synthesis mentioned herein after.

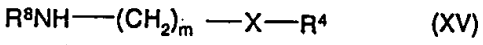
The starting compound of the Formula (IV) [wherein Y is -CONH-; R^4, R^8, and X are as defined above] can be prepared by condensation of the compound of the Formula (XIV),



(XIV)

[wherein R^7 is an amino protecting group, such as a Fmoc group, and R^8 is as defined above],

with a compound of the Formula (XV),



(XV)

[wherein R^4, R^8, X and m are as defined above],

followed by removal of the *tert*-butyl group. The compound of the Formula (XIV) is commercially available.

The starting compounds of the Formula (XV) [wherein X is a single bond, aryl, biphenyl or terphenyl group optionally containing one or more hetero atom(s) and/or being substituted with halogen atom(s) or lower alkyl] are commercially available or can be prepared by the methods similar to those described in EP 736 541 and Scheme 2: for example, $LiAlH_4$ reduction of the carboxyamide prepared from the carboxylic acid intermediates in Scheme 2 mentioned herein after, followed by protection of amino group with Fmoc chloride and the like.

The representative compounds of the Formula (IV) [wherein Y is -CONH- or -CON(lower alkyl)-; R^4, R^7, R^8 and X are as defined above] are

$HO_2C\text{-}CH_2CH(NHFmoc)\text{-}CONH\text{-}(CH_2)_{10}CH_3$,

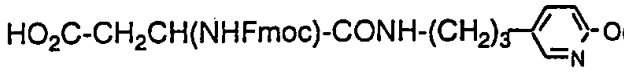
$HO_2C\text{-}CH_2CH(NHFmoc)\text{-}CONH\text{-}(CH_2)_{12}CH_3$,

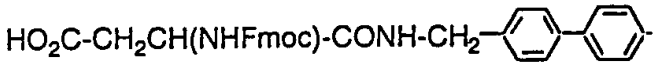
$HO_2C\text{-}CH_2CH(NHFmoc)\text{-}CONH\text{-}(CH_2)_{14}CH_3$,

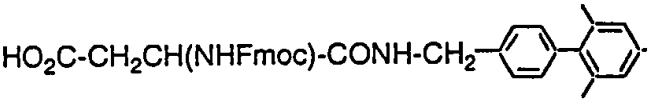
$HO_2C\text{-}CH_2CH(NHFmoc)\text{-}CONH\text{-}(CH_2)_{11}CH(CH_3)_2$,

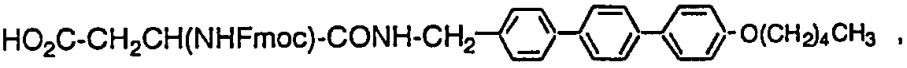
$HO_2C\text{-}CH_2CH(NHFmoc)\text{-}CONH\text{-}(CH_2)_{10}\text{-}CH{=}CH\text{-}CH_2CH_3$,

$HO_2C\text{-}CH_2CH(NHFmoc)\text{-}CONH\text{-}(CH_2)_8\text{-}CH{=}CH\text{-}(CH_2)_3CH_3$,







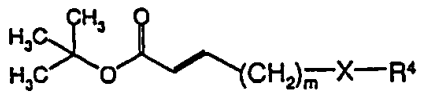


$HO_2C\text{-}CH_2CH(NHFmoc)\text{-}CON(CH_3)\text{-}(CH_2)_{12}CH_3$,

$HO_2C\text{-}CH_2CH(NHFmoc)\text{-}CON(CH_3)\text{-}(CH_2)_{14}CH_3$,

and the like.

The starting compound of the Formula (IV) [wherein Y is a single bond or $-CH_2-$; R^4, R^8, and X are as defined above] can be prepared by Michael addition of (R)-(+)-N-benzyl-1-phenylethylamine to a compound of the Formula (XVI),

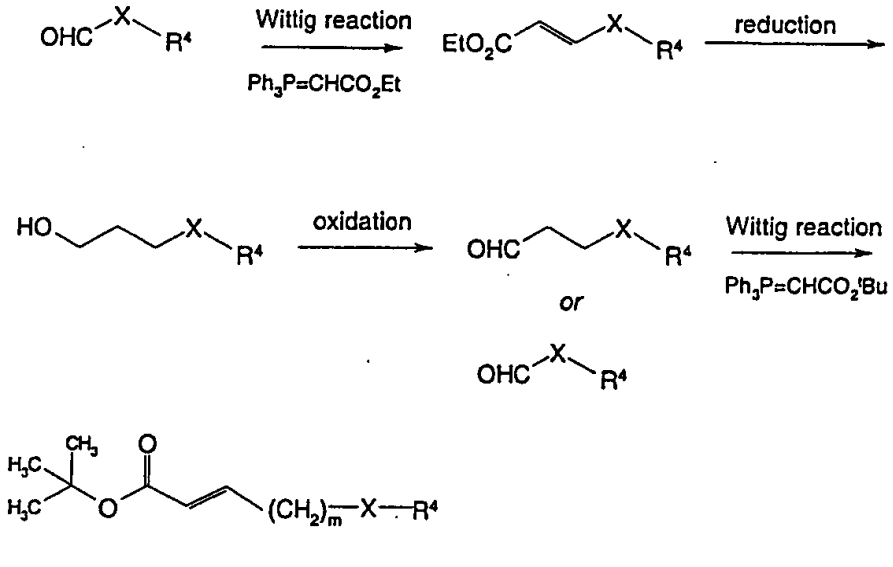


(XVI)

[wherein R^4, X and m are as defined above]

in the presence of strong base such as LDA [cf. Tetrahedron Asymmetry, 2 (3), 183 (1991)], followed by i) N-debenzylation by catalytic hydrogenation, ii) protection of the resulting primary amine with Fmoc chloride and the like, and iii) removal of *tert*-butyl group.

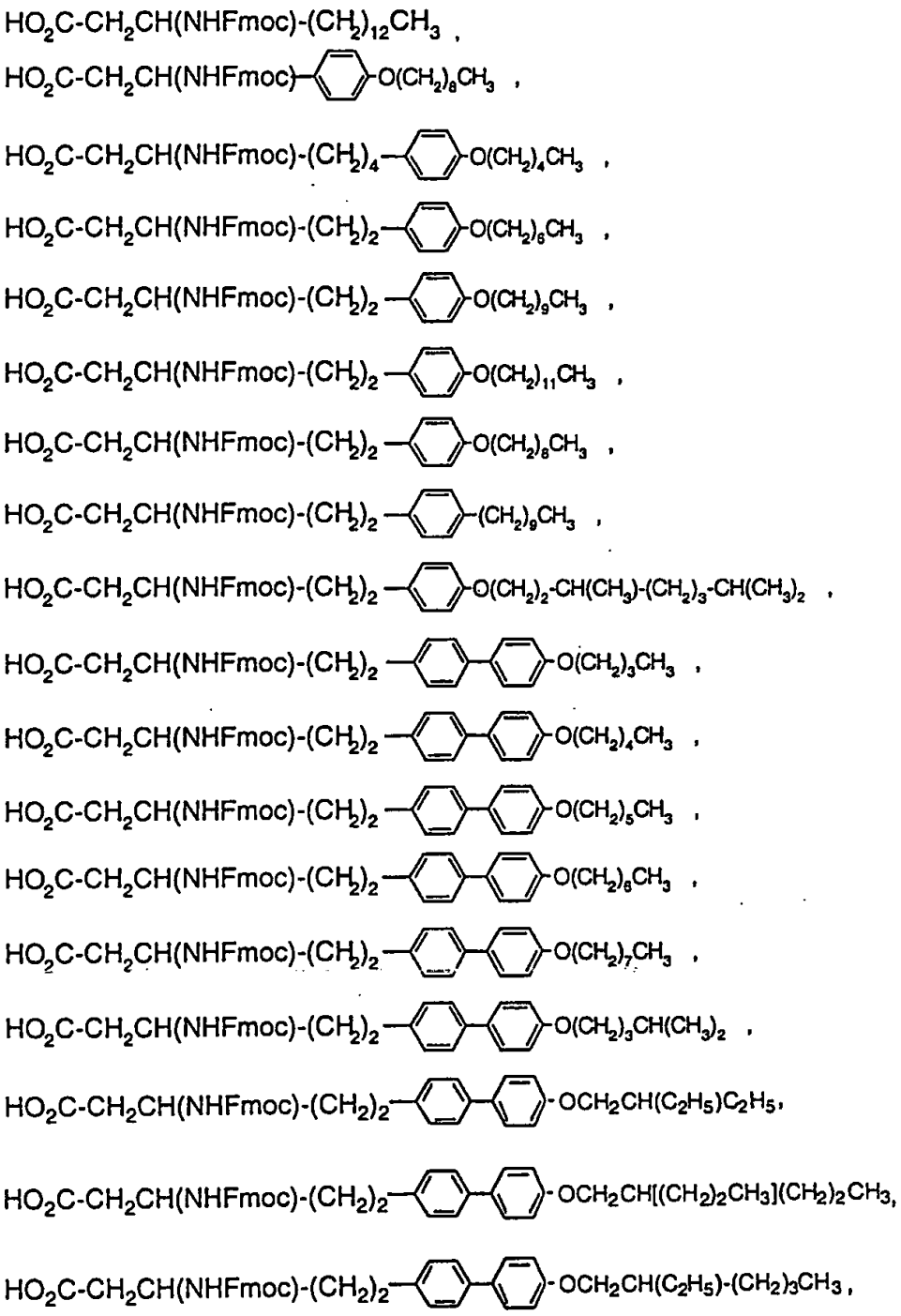
The starting compounds of the Formula (XVI) can be prepared by the method outlined in the following Scheme 2.

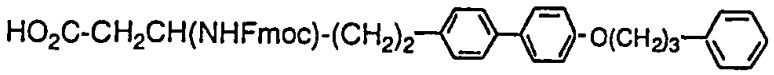


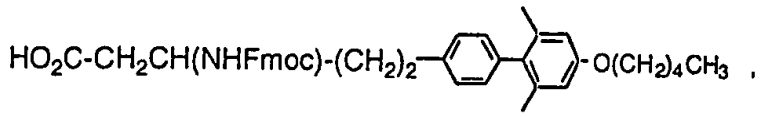
Scheme 2

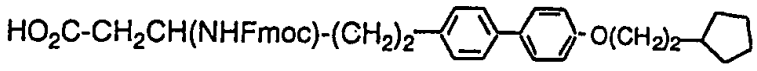
The compounds of the Formula (XVI), wherein m is 4, can be prepared by repeating the steps 1 to 3 in Scheme 2 before the last Wittig reaction.

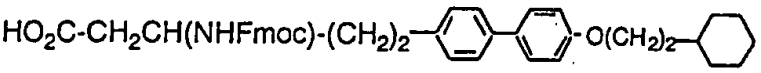
The representative compounds of the Formula (IV) [wherein Y is a single bond or $-CH_2-$; R^4, R^7, and X are as defined above] are:

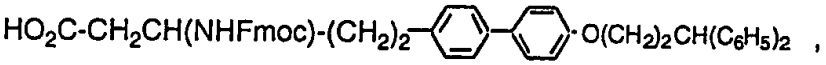


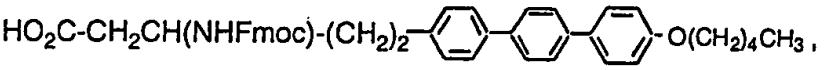


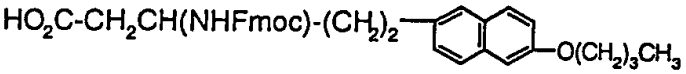


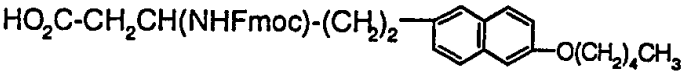


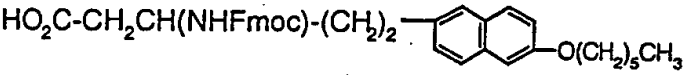


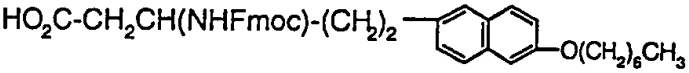


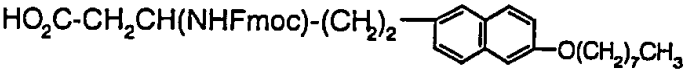


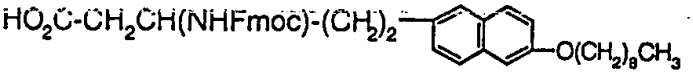


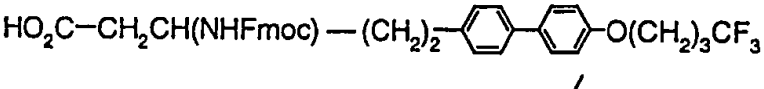


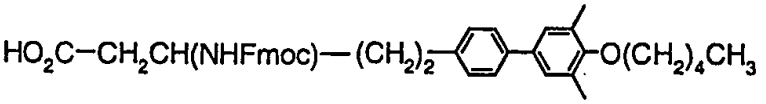












$HO_2C\text{-}CH_2CH(N(CH_3)Fmoc)\text{-}(CH_2)_{14}CH_3$

and the like.

The first peptide bond formation reaction as well as the cyclization of the resulting linear peptide can be performed by the method known to those skilled in the peptide chemistry [cf. The practice of Peptide Synthesis, M. Bodansky and A. Bodansky / 2nd ed., 1994 (Springer-Verlag)]. The preferable condensation agent is BOP-HOBt, PyBOP™-HOBt, PyBroP™-HOBt and the like [coupling reagents: commercially available (cf. The Combinatorial Chemistry Catalog, Feb., 1997; Novabiochem.)].

The reaction can be carried out in a solvent such as methanol, ethanol, pyridine, N,N-dimethylformamide, N-methylpyrrolidone and the like in the presence or absence of a base such as triethylamine, di-isopropylethylamine, pyridine and the like at a temperature between -20°C and +50°C, preferably at 0°C to +25°C.

Process C

Nitration of the Aerothricin of the Formula (I) can be performed by the method known to those skilled in the art; typically by sodium nitrite/acetic acid, tetranitromethane/pyridine and the like.

The reaction can be carried out at a temperature between -20° and 0°C, preferably at 0°C.

Process D

Reduction of nitro group(s) can be done by the method known to those skilled in the art; typically by catalytic hydrogenation using a catalyst such as palladium-C, platinum oxide and the like.

The reaction can be carried out at room temperature in a solvent such as methanol, ethanol, acetic acid, and the like.

Processes E and I

N-acylation of an amino group existing in R^1 or R^3 of the Formula (I) can be done with acid anhydride or carbamoyl chloride by the method known to those skilled in the art, or with carboxylic acid using condensation agents such as dicyclohexylcarbodiimide, BOP, HBTU, TNTU, PyBroP™, PyBOP™, TBTU, TSTU, HOBt and the like, or the combination of two of them.

The reaction can be carried out in a solvent such as methanol, ethanol, pyridine, N,N-dimethylformamide, N-methylpyrrolidone and the like in the presence or absence of a base such as triethylamine, di-isopropylethylamine, pyridine and the like at a temperature between -20°C and +50°C, preferably at 0°C to +25°C.

The removal of the amino protecting group, when using N-protected amino acid for the condensation reaction, can be done by the method known to those skilled in the art, e.g. treatment with trifluoroacetic acid for Boc group, or piperidine for Fmoc group.

Process F

N-monoalkylation of an amino group existing in R^1 of the Formula (I) can be done using acrylonitrile, ethoxymethylene-malononitrile or (1-ethoxyethylidene)malononitrile according to the method described in Organic Synthesis col. Vol. III, page 93, followed by reduction of the resulting nitrile group by catalytic hydrogenation or reduction with sodium borohydride/cobalt chloride, borane-methylsulide complex and the like [cf. J. Med. Chem., 37, 222 (1994)].

Process G

N-alkylation of the primary or secondary amino group existing in R^1 of the Formula (I) can be done by the conventional reductive alkylation with aldehyde derivatives of the Formula (V) using a reducing agent such as sodium cyanoborohydride in the presence or absence of weak acid such as acetic acid.

The reaction can be carried out at room temperature in a solvent such as methanol, ethanol, acetic acid and the like.

Process H

Examples of the compound (R^{12}-Q) of Formula (VI) for the substitution reaction are 2-bromo-5-nitropyridine, 2-chloropyrimidine, chloropyrazine and the like.

The substitution reaction can be carried out at a temperature between -20°C and +50°C, preferably at 0°C to +25°C, in a solvent such as acetonitrile, N,N-dimethylformamide and the like in the presence or absence of acid scavenger such as potassium carbonate, triethylamine, di-isopropylethyamine and the like.

Process J

The first mono-N-alkylation of an amino group existing in R^1 of the Formula (I) can be done by the method described in Process F. The successive N-acylation can be done by the method described in Process E and I.

Process K

The conversion of an amino group existing in R^1 of the Formula (I) into a guanidino group can be done by an activated amidine derivative such as 3,5-dimethyl-1H-pyrazole-1-carboxamidine, formamidinesulfonic acid, benztriazol-1-carboxamidinium tosylate and the like.

The reaction can be carried out in a solvent such as methanol, ethanol, water, N,N-dimethylformamide and the like at a temperature between 0°C and ~50°C, preferably at 20°C to ~30°C.

Process L

O-alkylation of a hydroxy group of the tyrosine residue in the Formula (I) can be done by the method known to those skilled in the art in the presence of acid scavenger such as sodium carbonate, diisopropylethylamine and the like [Can. J. Chem., 36, 1521 (1958)].

The reaction can be carried out in a solvent such as methanol, ethanol, acetone, N,N-dimethylformamide and the like at a temperature between 0°C and +50°C, preferably at 0°C to +25°C.

Process M

Iodination at the ortho position of the phenol group in a tyrosine residue can be done by treatment of Aerothricins of the Formula (I), wherein R^2 is hydrogen, with iodine monochloride or sodium iodide/aqueous sodium hypochlorite in a solvent such as methanol, ethanol and the like at room temperature.

The palladium(0) catalyzed coupling reaction with carbon monoxide, methyl acrylate and the like can be carried out using a palladium(0) catalyst such as $Pd(OAc)_2$, $Pd(OAc)_2(dppp)_2$ in a solvent such as methanol, ethanol, N,N-dimethylformamide,

acetonitrile and the like in the presence of base such as triethylamine at a temperature between 20°C and +100°C, preferably at 20°C to +70°C [Bioorg. Med. Chem. Lett., 7 (22), 2879 (1997)].

Process N

Dehydration of the carbamoyl group (R^5) of the Formula (I) can be done by Burgess reagent [available from Aldrich], cyanuric chloride, oxalyl chloride and the like [cf. J. Med. Chem., 37, 222 (1994)].

The reaction can be carried out in a solvent such as N,N-dimethylformamide, N-methylpyrrolidone and the like at room temperature.

Process O

The reduction of the carbamoyl or cyano group (R^5) of the Formula (I) can be done by sodium borohydride/cobalt chloride, borane-methylsulfide complex and the like [cf. J. Med. Chem., 37, 222 (1994)].

The reaction can be carried out in a solvent such as methanol, ethanol and the like at room temperature.

Process P

The hydroxysulfonation of the tyrosine residue of the Formula (I) can be carried out by sulfurtrioxide-DMF complex, sulfurtrioxide-pyridine complex or sulfurtrioxide-triethylamine complex in a solvent such as N,N-dimethylformamide, N-methylpyrrolidone, 1,4-dioxane, tetrahydrofuran and the like at a temperature between -30 to +70 °C, preferably at room temperature [cf. J. Chem. Soc. Perkin Trans, (6) 1739 (1990)].

Process Q

The reactions involved in this process can be done by the methods similar to those described in the process B - O.

The starting material, a linear peptide of the Formula (IX) can be obtained by cultivating a microorganism belonging to *Deuteromycotina* under aerobic conditions in an aqueous or a solid medium and isolating a linear peptide of Formula (IX) from the culture.

The microorganism used in the present invention can be any strains including mutants and variants belonging to *Deuteromycotina* capable of producing a linear peptide of Formula (IX). Especially preferred is strain NR 7379 which was isolated from fallen leaves collected at Kagoshima pref. in Japan, and identified as a strain belonging to *Deuteromycotina*.

The strain denoted as Deuteromycotina NR 7379 has been deposited with the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, Japan on June 16, 1998 under the Budapest Treaty as follows: Deuteromycotina NR 7379 (FERM BP-6391).

The cultivation in accordance with the process provided by the present invention can be carried out in a culture medium which contains customary nutrients usable by the microorganism being cultivated. As carbon sources there can be mentioned, for example, glucose, sucrose, starch, glycerol, molasses, dextrin and mixtures thereof. Nitrogen sources are, for example, soybean meal, cottonseed meal, meat extract, peptone, dried yeast, yeast extract, corn steep liquor, ammonium sulfate, sodium nitrate and mixtures thereof. Moreover, there may be added to the culture medium other organic or inorganic substances for promoting the growth of the microorganism and for increasing the production of a linear peptide of Formula (IX). Examples of such substances are inorganic salts such as, calcium carbonate, sodium chloride, phosphates and the like.

The cultivation is carried out under aerobic conditions preferably in a liquid medium by submerged fermentation, or in a solid medium by static fermentation. A temperature of 20°C to 30°C, with an optimal temperature of 27°C is suitable for cultivation. The cultivation is preferably carried out at a pH of 3 to 9. The cultivation time depends on the conditions under which the cultivation is carried out. In general, it is sufficient to carry out the cultivation for 120 to 672 h.

For harvesting the objective linear peptide of Formula (IX) from the cultures, separation methods which are usually employed to isolate metabolites produced by microbes from their cultures can be properly used. For example, a linear peptide of Formula (IX), which is a methanol extractable amphoteric substance, is recovered advantageously by the following procedures.

That is, the cultured broth obtained by liquid fermentation is extracted with an appropriate solvent to recover the proposed product. The solvents which can be used to extract the objective compound from the cultured broth include water-soluble organic solvents or hydrous solutions of water-soluble organic solvents, such as methanol, ethanol and hydrous alcohols, or water-immiscible organic solvent such as *n*-BuOH.

For removing salts, water soluble substances, etc. from the resulting extract, use is made of, with advantage, solvent partition between water and water-immiscible organic solvents, such as *n*-butanol, ethyl acetate, etc. For removing coloring substances, fat-soluble substance or the like from the extract, use is made of, with advantage, solvent purification by methanol, ethanol, a mixture of acetonitrile-0.1% aqueous trifluoroacetic acid, etc.

For complete purification of a linear peptide of Formula (IX), column chromatography is used with advantage. Carriers which can be used in such a column chromatography are such as Capcel Pak C18 UG80 (Shiseido Co. LTD, Japan). As an eluent, use is made of a solvent system consisting of a mixture of aqueous trifluoroacetic acid and appropriate water-soluble organic solvents such as methanol, ethanol, acetonitrile, etc. The eluate fraction thus purified, which contains a linear peptide of Formula (IX), can be subjected to concentration or freeze-drying to pulverize a linear peptide of Formula (IX).

A linear peptide of Formula (IX) was isolated as a trifluoroacetic acid salt, but the free linear peptide of Formula (IX) can be prepared by the following procedure. Namely, the linear peptide of Formula (IX) trifluoroacetic acid salt is dissolved in water, to which was added one equivalent of sodium hydroxide, and the mixture is subjected to Sephadex LH-20 column chromatography, followed by elution with a hydrous alcohol such as methanol-water, etc. to thereby obtain a linear peptide of Formula (IX).

The linear peptide of Formula (IX) provided by the present invention does not exhibit any fungicidal activity against various fungi, however, can be a key intermediate to produce potent antifungal agent such as Aerothricins.

The present invention is also concerned with acid addition salts of Aerothricins. The acid addition salt can be obtained as trifluoroacetic acid salt after normal course of isolation. The salt thus obtained may be dissolved in water and passed through an anion exchange column bearing the desired anion. The eluate containing the desired salt may be concentrated to recover the salt as a solid product.

The Aerothricins of Formula (I) may be converted to a corresponding salt by virtue of the presence of the tertiary nitrogen atoms.

The acid addition salt of Aerothricins of Formula (I) can be obtained by treatment of the free base of Aerothricins with at least a stoichiometric amount of an appropriate acid, such as mineral acids, e.g., hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like, and organic acids, e.g., acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, malic acid, malonic acid, succinic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid, and the like. Typically, the free base is dissolved in an inert organic solvent such as ethanol, methanol, and the like, and the acid added in a similar solvent. The temperature is maintained at about 40°C. The resulting salt precipitates spontaneously or may be brought out of solution with a less polar solvent.

The acid addition salts of the Aerothricins of Formula (I) may be converted to the corresponding free base by treatment with at least a stoichiometric amount of a suitable base such as sodium or potassium hydroxide, potassium carbonate, sodium bicarbonate, ammonia, and the like.

Aerothricins provided by the present invention exhibit broad fungicidal activity against various fungi and can be used as agents for treatment and prophylaxis of fungal infectious diseases. The *in vitro* and *in vivo* antifungal activity (see Tables 2 and 3) as well as the toxicity to hepatocytes (see Table 4) of the representative Aerothricins of Formula (I) are shown as follows:

1. *In vitro* antifungal activities

The *in vitro* antifungal activities of the representative Aerothricins of the present study were evaluated by determining the 50% inhibitory concentration (IC_{50}), which was calculated as the lowest concentration of an antifungal to inhibit the growth of fungus to 20% turbidity compared with the drug-free control growth spectrophotometrically.

The IC_{50} values were determined by the broth micro-dilution procedure based on NCCLS Approved Standard with the following minor modifications (National Committee for Clinical Laboratory Standards. (1997) Reference method for broth dilution antifungal susceptibility testing for yeasts. Approved standard. Document M27-A). Yeast Nitrogen Base (YNB; Difco Lab.) supplemented with 1% glucose and 0.25% K_2HPO_4 was used as testing medium for yeast, the same medium solidified with 0.2% low melting point agarose (BRL) was used for filamentous fungi. Inoculum size was 1-3 x 10^4 cells/ml, and incubation was performed for 1-2 days at 35°C.

Table 2: *In vitro* Antifungal activity, IC_{50} (μg/ml)

	Candida albicans CY1002	*Aspegillus fumigatus* CF1003	*Fusarium solani* CF1088
Aerothricin 1	0.03	0.06	0.21
Aerothricin 5	0.03	0.07	0.19
Aerothricin 12	0.09	0.10	2.20
Aerothricin 31	0.07	0.49	0.70
Aerothricin 36	0.08	0.05	1.00
Aerothricin 39	0.09	0.17	0.70
Aerothricin 41	0.08	0.03	2.40
Aerothricin 43	0.05	0.04	0.70
Aerothricin 45	0.07	0.08	2.30
Aerothricin 46	0.09	0.08	1.80
Aerothricin 47	0.09	0.11	1.40
Aerothricin 53	0.11	0.09	2.30
Aerothricin 54	0.15	0.17	0.74
Aerothricin 55	0.04	0.04	0.39
Aerothricin 57	0.14	0.05	1.30
Aerothricin 75	0.15	0.10	1.40
Aerothricin 77	0.13	0.10	0.67
Aerothricin 95	0.14	0.10	0.74

2. *In vivo* antifungal efficacy

In vivo antifungal efficacy of Aerothricins of the present invention is shown in the following Table 3. Mice of a conventional immunocompetent mouse strain, Crj: CD-1 (ICR) were used for experimental infection models of systemic candidiasis. 4 weeks old Crj: CD-1 (ICR) mice were used for systemic candidiasis by injecting *Candida albicans* $5x10^6$ conidia/mouse via the tail vein. Treatments were given twice (0, 4 h after infection) on the first day and once daily on following 2 days for systemic candidiasis (b.i.d x 1 day followed by q.d. x 2 days), intravenously (i.v.). 50% of effective dose (ED_{50}) values was calculated from the survival number at each dose on day 14.

Table 3: *In vivo* antifungal activity against systemic candidiasis in mice, ED_{50} (mg/kg) on day 14

Aerothricin 5	0.3
Aerothricin 16	0.3
Aerothricin 18	0.6
Aerothricin 36	0.6
Aerothricin 41	0.3
Aerothricin 42	0.6
Aerothricin 45	0.3
Aerothricin 46	0.4
Aerothricin 50	<0.3
Aerothricin 55	<0.3
Aerothricin 65	0.6

3. *In vitro* hepatotoxicity test

The mouse hepatocytes were isolated by a collagenase digestion and cultured in microtest plates. The hepatocyte monolayers were exposed to the test Aerothricins in the culture system for 1 day. After the culture period, the hepatocytes were observed under a microscope and evaluated morphologically. The degree of the morphological alteration (degeneration) of the hepatocytes by the test Aerothricins were compared with WF11243 and LY303366.

Table 4: Cytotoxicity to hepatocyte (μg/ml)

Aerothricin 14	>100
Aerothricin 15	>100
Aerothricin 21	>100
Aerothricin 34	>100
Aerothricin 38	>100
Aerothricin 45	>100
Aerothricin 47	>100
Aerothricin 48	>100
Aerothricin 53	>100
Aerothricin 65	>100
Aerothricin 67	>100
Aerothricin 72	>100
Aerothricin 81	>100
WF11243 (= Aerothricin 3)	100
LY303366	10

5 mg/kg and 30 mg/kg of Aerothricin 1 administration to mice for 4 weeks showed no acute toxicity.

Therefore, the novel Aerothricins of Formula (I) as well as pharmaceutically acceptable salts thereof exhibit potent antifungal activity against various fungal infections, including Aspergillosis, in mice over a wide range of dosages and are useful as antifungal agents. Moreover, the Aerothricins provided by this invention are much less cytotoxic to hepatocytes than the known cyclic peptide derivatives (WF11243 and LY303366).

Aerothricins of the present invention may also be useful for inhibiting or alleviating *Pneumocystis carinii* infections in immune-compromised patients.

The present invention further relates to the pharmaceutical compositions containing the novel Aerothricins of Formula (I) as well as pharmaceutically acceptable salts thereof.

The novel Aerothricins of Formula (I) as well as pharmaceutically acceptable salts thereof are highly active fungicidal agents. They are active against a variety of fungal species including *Candida spp.*, *Aspergillus spp.*, *Fusarium spp.*, *Mucor spp.* and *Absidia spp.*.

Thus, Aerothricins of the present invention are useful for topical and systemic treatment of mycoses in animals as well as humans. For example, they are useful in treating topical and mucosal fungal infections caused by *Candida spp.*, *Trichophyton spp.*, and *Microsporum spp.*. They may also be used in the treatment of systemic fungal infections caused by, for example, *Candida spp.*, *Aspergillus spp.*, or *Fusarium spp.*.

For clinical use, the novel Aerothricins of Formula (I) as well as pharmaceutically acceptable salts thereof can be administered alone, but will generally be administered in pharmaceutical admixture formulated as appropriate to the particular use and purpose desired, by mixing excipient, binding agent, lubricant, disintegrating agent, coating material, emulsifier, suspending agent, solvent, stabilizer, absorption enhancer and/or ointment base. The admixture can be used for oral, injectable, nasal, rectal or topical administration.

Pharmaceutical formulations of Aerothricins for oral administration may be granule, tablet, sugar coated tablet, capsule, pill, suspension or emulsion. For parenteral injection, for example, intravenously, intramuscularly or subcutaneously, Aerothricins of Formula (I) may be used in the form of a sterile aqueous solution which may contain other substances, for example, salts or glucose to make the solution isotonic. These compositions also may be presented in unit dosage form in ampoules or in multidose containers, preferable with added preservatives. Alternatively, the active ingredients may be in powder form for reconstituting with a suitable vehicle prior to administration. Aerothricins can also be administered in the form of a suppository or pessary, or they may be applied topically in the form of a lotion, solution, cream, ointment or dusting powder.

The daily dosage level of Aerothricins of Formula (I) is from 0.1 to 50 mg/kg (in divided doses) when administered by either the oral or parenteral route. Thus tablets or capsules of Aerothricins can be expected to contain from 5 mg to 0.5 g of active compound for administration singly or two or more at a time as appropriate. In any event the actual dosage can be determined by the physician and it may be varied upon the age, weight and response of the particular patient.

When Aerothricins are for antifungal use any method of administration may be employed. For treating mycotic infections, oral or intravenous administration is usually employed.

When Aerothricins are to be employed for control of pneumocystis infections it is desirable to directly treat lung and bronchi. For this reason inhalation methods are preferred. For administration by inhalation or nasal, Aerothricins of the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or nebulisers. The preferred delivery system for inhalation or nasal is a metered dose inhalation aerosol, which may be formulated as a powder, suspension or solution of a compound of Formula (I) in suitable propellants, such as fluorocarbons or hydrocarbons.

Although Aerothricins of the present invention may be employed as tablets, capsules, topical compositions, insufflation powders, suppositories, and the like, the solubility of Aerothricins of the present invention in water and aqueous media render them adaptable for use in injectable formulations and also in liquid compositions suitable for aerosol sprays.

The following Examples illustrate the preferred methods for the preparation of Aerothricins of the present invention, which are not intended to limit the scope of the invention thereto.

In the following Examples, the products were analyzed and purified by HPLC using a reverse phase column selected from those listed below. The mixed solvent consisted of 0.05% trifluoroacetic acid-water : 0.05% trifluoroacetic acid-acetonitrile with the appropriate ratio described in each working Example.

HPLC columns:

Column A:	CAPCELL PAK C18, UG-120, 4.6x250mm
Column B:	CAPCELL PAK C18, UG-120, 10x250mm
Column C:	CAPCELL PAK C18, UG-80, 20x250mm
Column D:	CAPCELL PAK C18, SG-120, 4.6x250mm
Column E:	CAPCELL PAK C18, SG-120, 10x250mm
Column F:	TSK GEL ODS-80Ts, 20x250mm

In the following working Examples, Aerothricins were obtained as trifluoroacetic acid salts unless otherwise indicated.

Reference Example 1

Preparation of (R)-3-(9-fluorenylmethoxycarbonylamino)-5-(4'-heptyloxybiphenyl-4-yl)-pentanoic acid

a) Preparation of 4-bromo-4'-heptyloxybiphenyl

To a stirred solution of 4-bromo-4'-hydroxybiphenyl (5.05 g, 20.2 mmol) in DMF (100 ml) were added K_2CO_3 (4.20 g, 30.4 mmol) and 1-bromoheptane (4.14 ml, 26.4 mmol), and then the mixture was heated at 80°C. After being stirred at 80°C for 20 h, the mixture was cooled to room temperature. The mixture was diluted with Et_2O (250 ml) and then the solution was washed with sat. brine (150 ml x 2). The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was recrystallized from CH_2Cl_2-petroleum ether to give 4-bromo-4'-heptyloxybiphenyl (6.21 g, 88%) as a white solid; FAB-MS: m/z 347[MH^+].

b) Preparation of 4-formyl-4'-heptyloxybiphenyl

To a cold (0°C) stirred solution of 4-bromo-4'-heptyloxybiphenyl (6.21 g, 17.9 mmol) in THF (120 ml) was added *n*-BuLi (1.66 M solution in hexane, 32.3 ml, 53.6 mmol). After the mixture was stirred at 0°C for 20 min., DMF (4.85 ml, 62.6 mmol) was added at -78°C. The mixture was stirred at -78°C for additional 20 min., and then quenched with sat. aqueous NH_4Cl. The mixture was diluted with EtOAc (220 ml), and then successively washed with sat. aqueous NH_4Cl. (125 ml) and sat. brine (100 ml). The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (EtOAc/hexane, 1:20) to give 4-formyl-4'-heptyloxybiphenyl (2.21 g, 42%) as a white amorphous powder.

c) Preparation of 3-(4'-heptyloxybiphenyl-4-yl)acrylic acid ethyl ester

To a stirred solution of 4-formyl-4'-heptyloxybiphenyl (2.21 g, 7.46 mmol) in benzene (40 ml) was added Ph_3P=CHCOOEt (5.19 g, 14.9 mmol), and then the mixture was heated at 60°C. After being stirred at 60°C for 3 h, the mixture was cooled to room

temperature and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (CH_2Cl_2/hexane, 1:2) to give 3-(4'-heptyloxybiphenyl-4-yl)acrylic acid ethyl ester (2.66 g, 97%) as a white amorphous powder.

FAB-MS: m/z 367[MH^+],

^{1}H NMR: δ 0.90 (t, *J*=6.8Hz, 3H), 1.25-1.55 (m, 8H), 1.35 (t, *J*=7.1Hz, 3H), 1.81 (quint, *J*=6.6Hz, 2H), 4.00 (t, *J*=6.4Hz, 2H), 4.28 (q, *J*=7.1Hz, 2H), 6.46 (d, *J*=16.0Hz, 1H), 6.94-7.00 (m, 2H), 7.50-7.60 (m, 6H), 7.72 (d, *J*=16.0Hz, 1H).

d) Preparation of 3-(4'-heptyloxybiphenyl-4-yl)propionic acid ethyl ester

To a stirred solution of 3-(4'-heptyloxybiphenyl-4-yl)acrylic acid ethyl ester (2.65 g, 7.23 mmol) in CH_2Cl_2 (60 ml) was added palladium on activated carbon (Pd ca.10wt%, 1.07 g), and then the mixture was set under H_2 atmosphere. After being stirred for 2 h, the mixture was filtered through a pad of Celite and washed with CH_2Cl_2. Filtrate and washings were combined and concentrated *in vacuo* to give 3-(4'-heptyloxybiphenyl-4-yl)propionic acid ethyl ester (crude, 2.74 g) which was used for the next step without further purification.

^{1}H NMR: δ 0.90 (t, *J*=6.6Hz, 3H), 1.25 (t, *J*=7.3Hz, 3H), 1.29-1.56 (m, 8H), 1.75-1.86 (m, 2H), 2.65 (t, *J*=7.8Hz, 2H), 2.98 (t, *J*=7.8Hz, 2H), 3.99 (t, *J*=6.6Hz, 2H), 4.14 (q, *J*=7.3Hz, 2H), 6.93-6.98 (m, 2H), 7.25 (d, *J*=8.6Hz, 2H), 7.43-7.52 (m, 4H).

e) Preparation of 3-(4'-heptyloxybiphenyl-4-yl)propan-1-ol

To a cold (0°C) stirred suspension of LiAlH4 (0.47 g, 12.4 mmol) in THF (20 ml) was added a solution of 3-(4'-heptyloxybiphenyl-4-yl)propionic acid ethyl ester (crude, 2.74 g) in THF (30 ml). After being stirred for 30 min. at room temperature, the mixture was quenched with H_2O at 0°C. The mixture was filtered through a pad of Celite and washed with CH_2Cl_2. The filtrate and washings were combined and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (EtOAc/hexane, 2:3) to give 3-(4'-heptyloxy-biphenyl-4-yl)propan-1-ol (2.27 g, 96% for 2 steps) as a white amorphous powder.

EI-MS: m/z 326[M^+],

^{1}H NMR: δ 0.90 (t, *J*=6.8Hz, 3H), 1.21-1.55 (m, 8H), 1.81 (quint, *J*=6.6Hz, 2H), 1.86-2.00 (m, 2H), 2.75 (t, *J*=7.3Hz, 2H), 3.71 (t, *J*=6.6Hz, 2H), 3.99 (t, *J*=6.6Hz, 2H), 6.92-7.00 (m, 2H), 7.25 (d, *J*=7.9Hz, 2H), 7.44-7.55 (m, 4H).

f) Preparation of 3-(4'-heptyloxybiphenyl-4-yl)propionaldehyde

To a cold (0°C) stirred solution of 3-(4'-heptyloxybiphenyl-4-yl)propan-1-ol (2.26 g, 6.92 mmol) in CH_2Cl_2 (60 ml) were added molecular sieves 4A powder (5.17 g) and PCC (5.25 g, 24.4 mmol). After being stirred for 2 h at room temperature, Et_2O (20 ml) was added to the mixture. The reaction mixture was transferred to a short silica gel column and eluted with CH_2Cl_2. The eluate was concentrated *in vacuo* to give 3-(4'-heptyloxybiphenyl-4-yl)propionaldehyde (crude, 2.45 g) which was used for the next step without further purification.

g) Preparation of 3-(4'-heptyloxybiphenyl-4-yl)pent-2-enoic acid *tert*-butyl ester

To a stirred solution of 3-(4'-heptyloxybiphenyl-4-yl)propionaldehyde (crude, 2.45 g) in benzene (150 ml) was added Ph_3P=CHCOO*t*-Bu (5.21 g, 13.8 mmol), and then the mixture was heated at 60°C. After being stirred for 30 min. at 60°C, the mixture was cooled to room temperature and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (EtOAc/hexane, 1:30) to give 3-(4'-heptyloxybiphenyl-4-yl)pent-2-enoic acid *tert*-butyl ester (1.95 g, 67% for 2 steps) as a white amorphous powder.

EI-MS: m/z 422[M^+],

^{1}H NMR: δ 0.90 (t, *J*=6.6Hz, 3H), 1.21-1.51 (m, 8H), 1.49 (s, 9H), 1.74-1.87 (m, 2H), 2.47-2.58 (m, 2H), 2.79 (t, *J*=7.3Hz, 2H), 3.99 (t, *J*=6.6Hz, 2H), 5.81 (d.t., *J*=1.5Hz, 15.5Hz, 1H), 6.87-7.01 (m, 3H), 7.23 (d, *J*=7.9Hz, 2H), 7.44-7.53 (m, 4H).

h) Preparation of (R)-3-[benzyl-((R)-1-phenylethyl)amino]-5-(4'-heptyloxybiphenyl-4-yl)pentanoic acid *tert*-butyl ester

To a cold (0°C) stirred suspension of (R)-N-benzyl-1-phenylethylamine hydrochloride (3.28 g, 13.2 mmol) in THF (40 ml) was added *n*-BuLi (1.61 M solution in hexane, 15.0 ml, 24.2 mmol). After the mixture was stirred for 25 min. at 0°C, a solution of 3-(4'-heptyloxybiphenyl-4-yl)pent-2-enoic acid *tert*-butyl ester (1.94 g, 4.38 mmol) in THF

(30 ml) was added at -78°C. After the mixture was stirred for additional 20 min. at -78°C, the reaction mixture was quenched with sat. aqueous NH_4Cl. and concentrated *in vacuo*. The residue was diluted with sat. aqueous NH_4Cl. (200 ml), and then extracted with CH_2Cl_2 (200 ml x 2). The combined extracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (EtOAc/hexane, 1:40) to give (R)-3-[benzyl-((R)-1-phenylethyl)amino]-5-(4'-heptyloxybiphenyl-4-yl)pentanoic acid *tert*-butyl ester (2.83 g, quant.) as a colorless oil.

EI-MS: m/z 633[M^+],

^{1}H NMR: δ 0.91 (t, *J*=6.6Hz, 3H), 1.24-1.55 (m, 13H), 1.38 (s, 9H), 1.57-2.04 (m, 6H), 2.52-2.69 (m, 1H), 2.97-3.10 (m, 1H), 3.37-3.49 (m, 1H), 3.55 (ABq, *J*=15.0 Hz, 1H), 3.85 (ABq, *J*=15.0Hz, 1H), 3.88 (q, *J*=6.9Hz, 1H), 4.00 (t, *J*=6.6Hz, 1H), 6.96 (d, *J*=8.6Hz, 2H), 7.16 (d, *J*=8.2Hz, 2H), 7.21-7.53 (m, 16H).

i) Preparation of (R)-3-amino-5-(4'-heptyloxybiphenyl-4-yl)pentanoic acid *tert*-butyl ester

To a stirred solution of (R)-3-[benzyl-((R)-1-phenylethyl)amino]-5-(4'-heptyloxybiphenyl-4-yl)pentanoic acid *tert*-butyl ester (2.82 g, 4.45 mmol) in EtOAc (50 ml) were added AcOH (2.5 ml) and $Pd(OH)_2$ on carbon ($Pd(OH)_2$ ca. 20wt%, 1.07 g), and then the mixture was set under H_2 atmosphere. After being stirred for 15 h, the mixture was filtered through a pad of Celite and washed with MeOH. The filtrate and washings were combined, and concentrated *in vacuo* to give (R)-3-amino-5-(4'-heptyloxybiphenyl-4-yl)pentanoic acid *tert*-butyl ester (crude, 3.14 g) which was used for the next step without further purification.

j) Preparation of (R)-3-(9-fluorenylmethoxycarbonylamino)-5-(4'-heptyloxybiphenyl-4-yl)pentanoic acid *tert*-butyl ester

To a stirred suspension of (R)-3-amino-5-(4'-heptyloxybiphenyl-4-yl)pentanoic acid *tert*-butyl ester (crude, 3.14 g) in 50% aqueous 1,4-dioxane (40 ml) were added Na_2CO_3 (1.19 g, 11.2 mmol) and FmocCl (1.28 g, 4.95 mmol). After being stirred for 1 h, the mixture was diluted with sat. brine (100 ml) and extracted with CH_2Cl_2 (100 ml x 3). The combined extracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give (R)-3-(9-fluorenylmethoxycarbonylamino)-5-(4'-heptyloxybiphenyl-4-yl)pentanoic acid tert-butyl ester (crude, 3.34 g) which was used for the next step without further purification.

FAB-MS: m/z 668[M^++Li],

^{1}H NMR: δ 0.81 (t, *J*=6.6Hz, 3H), 1.15-1.44 (m, 8H), 1.35 (s, 9H), 1.62-1.93 (m, 4H), 2.29-2.68 (m, 4H), 3.84-4.02 (m, 1H), 3.88 (t, *J*=6.6Hz, 2H), 4.13 (t, *J*=6.8Hz, 1H), 4.25-4.41 (m, 2H), 5.27 (d, *J*=9.2Hz, 1H), 6.85 (d, *J*=8.6Hz, 2H), 7.06-7.42 (m, 10H), 7.51 (d, *J*=7.3Hz, 2H), 7.66 (d, *J*=7.6Hz, 2H).

k) Preparation of (R)-3-(9-fluorenylmethyloxycarbonylamino)-5-(4'-heptyloxybiphenyl-4-yl)pentanoic acid

To a stirred solution of (R)-3-(9-fluorenylmethoxycarbonyl-amino)-5-(4'-heptyloxybiphenyl-4-yl)pentanoic acid *tert*-butyl ester (crude, 3.34 g) in CH_2Cl_2 (20 ml) was added TFA (20 ml) dropwise. After being stirred for 1 h at room temperature, the mixture was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (MeOH/CH_2Cl_2, 1:20) to give (R)-3-(9-fluorenylmethoxycarbonylamino)-5-(4'-heptyloxybiphenyl-4-yl)pentanoic acid (2.07 g, 77% in 3 steps) as a white amorphous powder.

FAB-MS: m/z 606[MH^+],

^{1}H NMR: δ 0.88 (t, *J*=6.6Hz, 3H), 1.21-1.51 (m, 8H), 1.64-2.04 (m, 2H), 1.78 (q, *J*=6.6Hz, 2H), 2.27-2.78 (m, 4H), 3.91-4.07 (m, 1H), 3.96 (t, *J*=6.6Hz, 2H), 4.20 (t, *J*=6.6Hz, 1H), 4.34-4.56 (m, 2H), 5.09-5.28 (m, 1H), 6.92 (d, *J*=8.9Hz, 2H), 7.10-7.49 (m, 10H), 7.57 (d, *J*=7.3Hz, 2H), 7.73 (d, *J*=7.3Hz, 2H).

The starting compounds of Formula (IV) [wherein Y is a single bond or -CH_2-] used in the process B were prepared according to the method similar to that described above.

Reference Example 2

Preparation of (S)-3-(9H-fluorenylmethoxycarbonylamino)-N-undecylsuccinamic acid

a) To a solution of (S)-2-(9H-fluoren-9-ylmethoxycarbonyl-amino)succinic acid (150 mg, 0.36 mmol), BOP reagent (162 mg, 0.36 mmol) and HOBT hydrate (56 mg, 0.36 mmol) in N,N-dimethylformamide (0.2 ml) was added N,N-diisopropylethylamine (64 µl, 0.36 mmol). After being stirred for 30 min at room temperature, 1-aminoundecane (79 µl,

0.37 mmol) was added. The mixture was stirred at room temperature for 3 h. The reaction mixture was diluted with water and extracted with Et_2O. The combined extracts were washed with water, dried over anhydrous sodium sulfate, filtered and concentrated. Purification of the residue by silica gel column chromatography (using *n*-hexane:ethyl acetate = 3:1 as an eluent) gave (S)-3-(9H-fluoren-9-ylmethoxycarbonylamino)-N-undecylsuccinamic acid *tert*-butyl ester (169 mg, 82% yield) as a colorless amorphous solid.

FAB-MS (m/z): 565[MH^+],

^{1}H-NMR($CDCl_3$) δ: 0.88 (3H, t, *J*=7Hz), 1.24 (16H, m), 1.45 (11H, m), 2.58 (1H, dd, J_1=17Hz, J_2=7Hz), 2.91 (1H, dd, J_1=17Hz, J_2=4Hz), 3.23 (2H, q, *J*=7Hz), 4.22 (1H, t, *J*=7Hz), 4.42~4.45 (3H, m), 5.94 (1H, broad d, *J*=8Hz), 6.43 (1H, broad s), 7.31 (2H, t, *J*=7Hz), 7.41 (2H, t, *J*=7Hz), 7.58 (2H, d, *J*=7Hz), 7.77 (2H, d, *J*=7Hz).

b) A solution of (S)-3-(9H-fluoren-9-ylmethoxycarbonylamino)-N-undecylsuccinamic acid *tert*-butyl ester (113 mg, 0.2 mmol) in TFA (2 ml) was stirred at room temperature for 30 min. After completion of the reaction, TFA was removed by evaporation *in vacuo*. Purification of the residue by silica gel column chromatography (using dichloromethane:methanol = 9:1 as an eluent) gave (S)-3-(9H-fluoren-9-ylmethoxycarbonylamino)-N-undecylsuccinamic acid (101 mg, 99% yield) as a colorless amorphous solid.

FAB-MS (m/z): 507[MH^+],

^{1}H-NMR($CDCl_3$) δ: 0.87 (3H, t, *J*=7Hz), 1.23 (16H, m), 1.46 (2H, m), 2.62~2.80 (1H, m), 2.90~3.05 (1H, m), 3.21 (2H, m), 4.20 (1H, t, *J*=7Hz), 4.44 (2H, d, *J*=6Hz), 4.53 (1H, broad s), 5.98 (1H, m), 6.52 (1H, broad s), 7.30 (2H, t, *J*=7Hz), 7.40 (2H, t, *J*=7Hz), 7.56 (2H, d, *J*=7 Hz), 7.76 (2H, d, *J*=7Hz).

The starting compounds of the general Formula (IV) [wherein Y is -CONH- or -CON(CH_3)-] used in the process B were prepared according to the method described above.

combined, frozen and lyophilized to give 49.5 mg of the linear peptide C, a precursor for cyclization, as a white amorphous solid.

To a stirred solution of the linear peptide C (49.5 mg, 0.029 mmol) obtained above in DMF (27 ml) was added HOBT hydrate (11.3 mg, 0.074 mmol), N,N-diisopropyletylamine (0.018 ml, 0.105 mmol) and a solution of BOP reagent (33.1 mg, 0.075 mmol) in DMF (4 ml). After the mixture was stirred for 3 h at room temperature, the solvent was evaporated *in vacuo*.

The residue obtained above was dissolved in TFA (6 ml), and stirred at 0° C for 30 min. TFA was then evaporated *in vacuo*. The residue was purified by preparative reverse phase HPLC, the detailed condition of which is shown below. The appropriate fractions were combined, frozen and lyophilized to give 19.4 mg of Aerothricin 33 as a white amorphous solid.

HPLC(Rt): 12.4 min. (column C, flow rate: 9 ml/min.; eluent: 0.05% trifluoroacetic acid-water : 0.05% trifluoroacetic acid-acetonitrile = 61:39); FAB-MS (m/z): 1568[MH^+].

The following Aerothricins 34-38, 40-53, 64-73 and 89-95, 97-99 and 123 were prepared according to the method similar to that descried in this Example 8 using the corresponding building block represented as Formula (IV).

	FAB-MS	HPLC	Analytical condition
Compound name	m/z: [MH^+]	retention time (min.)	(column) (flow rate; ratio of eluent*)
Aerothricin 34	1568	14.1	(C)(9 ml/min.; 60/40)
Aerothricin 35	1568	13.2	(C)(9 ml/min.; 57/43)
Aerothricin 36	1610	21.9	(C)(9 ml/min.; 55/45)
Aerothricin 37	1638	44.1	(C)(9 ml/min.; 54/46)
Aerothricin 38	1610	28.1	(C)(9 ml/min.; 58/42)
Aerothricin 40	1602	16.8	(F)(10 ml/min.; 57/43)
Aerothricin 41	1616	20.6	(C)(9 ml/min.; 60/40)
Aerothricin 42	1630	16.8	(F)(10 ml/min.; 62/38)
Aerothricin 43	1644	29.2	(C)(9 ml/min.; 57/43)
Aerothricin 44	1658	35.5	(F)(10 ml/min.; 50/50)
Aerothricin 45	1630	24.7	(C)(9 ml/min.; 59/41)
Aerothricin 46	1664	18.7	(C)(9 ml/min.; 59/41)
Aerothricin 47	1594	22.9	(C)(9 ml/min.; 54/46)
Aerothricin 48	1576	24.4	(F)(10 ml/min.; 58/42)
Aerothricin 49	1590	24.2	(C)(9 ml/min.; 65/35)

Aerothricin 50	1604	48.9	(F)(10 ml/min.; 55/45)
Aerothricin 51	1618	40.4	(F)(9 ml/min.; 60/40)
Aerothricin 52	1632	32.5	(F)(10 ml/min.; 50/50)
Aerothricin 53	1646	27.0	(C)(9 ml/min.; 54/46)
Aerothricin 64	1547	15.5	(B)(4 ml/min.; 65/35)
Aerothricin 65	1575	15.5	(C)(9 ml/min.; 55/45)
Aerothricin 66	1603	16.6	(C)(9 ml/min.; 52/48)
Aerothricin 67	1587	19.9	(C)(9 ml/min.; 59/41)
Aerothricin 68	1587	19.6	(C)(9 ml/min.; 59/41)
Aerothricin 69	1589	21.8	(C)(9 ml/min.; 58/42)
Aerothricin 70	1617	21.6	(C)(9 ml/min.; 53/47)
Aerothricin 71	1746	30.0	(C)(9 ml/min.; 64/36)
Aerothricin 72	1673	22.6	(C)(9 ml/min.; 57/43)
Aerothricin 73	1721	20.2	(C)(9 ml/min.; 55/45)
Aerothricin 89	1630	22.1	(F)(10 ml/min.; 55/45)
Aerothricin 90	1658	24.9	(F)(10 ml/min.; 50/50)
Aerothricin 91	1670	26.7	(F)(10 ml/min.; 50/50)
Aerothricin 92	1642	26.0	(F)(10 ml/min.; 55/45)
Aerothricin 93	1650	21.4	(F)(10 ml/min.; 57/43)
Aerothricin 94	1658	30.8	(F)(10 ml/min.; 52/48)
Aerothricin 95	1574	28.3	(C)(9 ml/min.; 57/43)
Aerothricin 97	1740	44.7	(F)(10 ml/min.; 57/43)
Aerothricin 98	1656	30.0	(F)(10 ml/min.; 62/38)
Aerothricin 99	1644	16.9	(F)(10 ml/min.;53/47)
Aerothricin 123	1630	20.7	(F)(10 ml/min.;56/44)

* ratio of 0.05% trifluoroacetic acid-water : 0.05% trifluoroacetic acid-acetonitrile

Example 9

Preparation of Aerothricin 16

(a). To a stirred solution of Compound A (described in Reference Example 3) (1 g, 0.61 mmol) in pyridine (2.5 ml) was added tetranitromethane (0.365 ml, 3.05 mmol). After being stirred for 4 h at room temperature, the reaction mixture was concentrated *in vacuo*. The dark-brown residue was purified by reverse phase HPLC (Lobar RP18, 10 ml/min., 0.05% trifluoroacetic acid-water : 0.05% trifluoroacetic acid-acetonitrile = 50:50 → 33:66 0.05% TFA). The appropriate fractions were combined, frozen and lyophilized to give 711 mg of the crude nitro derivative of Compound A as a pale yellow amorphous solid.

(b). A mixture of the crude product obtained above (12 mg, 0.0071 mmol) and TFA (0.5 ml) was stirred at 0°C for 30 min. TFA was evaporated under a stream of dry nitrogen.

The yellow residue was purified by preparative reverse phase HPLC. The appropriate fractions were combined, frozen and lyophilized to give 8 mg of Aerothricin 16·TFA salt as a pale yellow amorphous solid.

HPLC(Rt): 15.5 min. (column B, flow rate: 4 ml/min., eluent: 0.05% trifluoroacetic acid-water : 0.05% trifluoroacetic acid-acetonitrile = 55:45); FAB-MS (m/z): 1578[MH^+].

The following Aerothricins 39, 54, 55 and 77 were prepared according to the method similar to that described in Example 9, using Aerothricins obtained in Example 8 as the starting material.

Compound name	FAB-MS m/z: [MH^+]	HPLC retention time (min.)	Analytical condition (column) (flow rate; ratio of eluent*)
Aerothricin 39	1577	13.2	(C)(9 ml/min.; 55/45)
Aerothricin 54	1661	14.2	(C)(9 ml/min.; 57/43)
Aerothricin 55	1689	27.8	(C)(9 ml/min.; 55/45)
Aerothricin 77	1648	25.0	(C)(9 ml/min.; 53/47)

* ratio of 0.05% trifluoroacetic acid-water : 0.05% trifluoroacetic acid-acetonitrile

Example 10

Preparation of Aerothricin 17

(a). To the solution of the crude nitro derivative of Compound A, obtained in Example 9(a), (55 mg, 0.033 mmol) in MeOH (5 ml) was added 10% palladium on charcoal (20 mg), and the reaction vessel was filled with hydrogen. After being stirred for 13.5 h at room temperature, the mixture was filtered through membrane filter (pore size: 0.2 μm) and the solvent was evaporated to give 52 mg of the crude amino derivative of Aerothricin 3 as brown amorphous, which was used in the next step without further purification.

(b). A mixture of the crude amino derivative of Compound A (described in Reference Example 3), obtained above, (3.4 mg, 0.0021 mmol) and TFA (0.2 ml) was stirred at 0°C for 30 min. TFA was evaporated under a stream of dry nitrogen. The brown

residue was purified by preparative reverse phase HPLC. The appropriate fractions were combined, frozen and lyophilized to give 1.3 mg of Aerothricin 17 as a colorless amorphous solid.

HPLC(Rt): 12.8 min. (column A, flow rate: 1 min./ml, eluent: 0.05% trifluoroacetic acid-water : 0.05% trifluoroacetic acid-acetonitrile = 59:41); FAB-MS (m/z): 1548[MH^+].

The following Aerothricins 29, 56 and 78 were prepared according to the method similar to that described in Example 10, using Aerothricins obtained in Example 9 as the starting material.

	FAB-MS	HPLC	Analytical condition
Compound name	m/z: [MH^+]	retention time (min.)	(column) (flow rate; ratio of eluent*)
Aerothricin 29	1606	31.0	(C)(9 ml/min.; 60/40)
Aerothricin 56	1659	15.1	(C)(9 ml/min.; 57/43)
Aerothricin 78	1618	16.8	(C)(9 ml/min.; 57/43)

* ratio of 0.05% trifluoroacetic acid-water : 0.05% trifluoroacetic acid-acetonitrile

Example 11

Preparation of Aerothricin 18

(a). To a solution of the crude amino derivative of Compound A, obtained in Example 10(a), (1.7 mg, 0.001 mmol) in methanol (0.05 ml) and pyridine (0.025 ml) was added Boc-Gly-OH (18 mg, 0.10 mmol), WSCI (30 mg, 0.15 mmol) and HOBT hydrate (24 mg, 0.15 mmol) successively. After the mixture was stirred for 15 h at room temperature, the solvent was removed by a stream of dry nitrogen.

(b). The crude residue obtained above was dissolved in TFA (0.1 ml) and stirred at 0°C for 30 min. TFA was removed with a stream of dry nitrogen. The residue was purified by preparative reverse phase HPLC. The appropriate fractions were combined, frozen and lyophilized to give 0.54 mg of Aerothricin 18 as a colorless amorphous solid.

HPLC(Rt): 8.9 min. (column B, flow rate: 4 ml/min., eluent: 0.05% trifluoroacetic acid-water : 0.05% trifluoroacetic acid-acetonitrile = 57:43); FAB-MS (m/z): 1605[MH^+].

The following Aerothricins 19-23, 30, 57-62, 79, and 81 were prepared according to the method similar to that described in Example 11 using the corresponding acylating agent and Aerothricins obtained in Example 10 as the starting material.

	FAB-MS	HPLC	Analytical condition
Compound name	m/z: [MH^+]	retention time (min.)	(column) (flow rate; ratio of eluent*)
Aerothricin 19	1590	17.5	(A)(1 ml/min.; 57/43)
Aerothricin 20	1619	6.0	(B)(4 ml/min.; 55/45)
Aerothricin 21	1663	18.0	(C)(9 ml/min.; 60/40)
Aerothricin 22	1605	12.5	(A)(1 ml/min.; 55/45)
Aerothricin 23	1620	23.9	(C)(9 ml/min.; 55/45)
Aerothricin 30	1676	24.6	(C)(9 ml/min.; 61/39)
Aerothricin 57	1701	21.2	(C)(9 ml/min.; 56/44)
Aerothricin 58	1730	23.4	(C)(9 ml/min.; 55/45)
Aerothricin 59	1716	13.7	(C)(9 ml/min.; 58/42)
Aerothricin 60	1730	16.3	(C)(9 ml/min.; 55/45)
Aerothricin 61	1730	39.1	(C)(9 ml/min.; 47/53)
Aerothricin 62	1730	15.8	(C)(9 ml/min.; 55/45)
Aerothricin 79	1689	36.1	(C)(9 ml/min.; 57/43)
Aerothricin 81	1675	24.4	(C)(9 ml/min.; 60/40)

* ratio of 0.05% trifluoroacetic acid-water : 0.05% trifluoroacetic acid-acetonitrile

Example 12

Preparation of Aerothricin 12

To a solution of Aerothricin 5 (7.5 mg, 0.0048 mmol), 37% formalin (150 µl) and acetic acid (50 µl) in MeOH (1.0 ml) was added sodium cyanoborohydride (7.5 mg, 0.119 mmol) in MeOH (100 µl) at room temperature and the mixture was stirred for 7 h at room temperature. After the solvent was evaporated *in vacuo*, the residue was dissolved in *n*-butanol and washed with diluted hydrochloric acid and water successively. The organic layer was evaporated *in vacuo*. The residue was purified by preparative reverse phase HPLC, the detailed condition of which is shown below. The appropriate fractions were combined, frozen and lyophilized to give 5.4 mg of Aerothricin 12 as a colorless amorphous solid.

HPLC(Rt): 7.1 min. (column B, flow rate: 4 ml/min., eluent: 0.05% trifluoroacetic acid-water : 0.05% trifluoroacetic acid-acetonitrile = 50:50); FAB-MS (m/z): 1575[MH^+].

The following Aerothricins 13, 25, 30 and 75 were prepared according to the method similar to that described in Example 12.

	FAB-MS	HPLC	Analytical condition
Compound name	m/z: [MH^+]	retention time (min.)	(column) (flow rate; ratio of eluent*)
Aerothricin 13	1561	13.7	(B)(4 ml/min.; 55/45)
Aerothricin 25	1607	23.5	(C)(4 ml/min.; 55/45)
Aerothricin 30	1676	24.6	(C)(9 ml/min.; 61/39)
Aerothricin 75	1631	24.2	(C)(9 ml/min.; 55/45)

* ratio of 0.05% trifluoroacetic acid-water : 0.05% trifluoroacetic acid-acetonitrile

Example 13

Prepration of Aerothricin 111

(a). To a solution of Aerothricin 3 (500 mg, 0.326 mmol), (2-oxoethyl)-carbamic acid *tert*-butyl ester* (1.66g, 10.4 mmol) and acetic acid (5 ml) in MeOH (45 ml) was added sodium cyanoborohydride (410 mg, 6.52 mmol) in MeOH (5 ml) at room temperature. The mixture was stirred for 18 h at room temperature. After the solvent was evaporated *in vacuo*, the residue was dissolved in *n*-butanol and washed with diluted hydrochloric acid and water successively. The organic layer was evaporated *in vacuo*.

The crude residue was used for the next step without further purification.

*CAS No. 89711-08-0

(b). A solution of the crude residue obtained above in TFA (20 ml) was stirred at 0°C for 30 min. TFA was evaporated *in vacuo*. The residue was purified by preparative reverse HPLC, the detailed condition of which is shown below. The appropriate fraction were combined, frozen and lyophilized to give 253 mg of Aerothricin 111 as a colorless amorphous solid.

HPLC(Rt) 18.6 min. (column F, flow rate: 10ml/min., eluent: 0.05% trifluoroacetic acid-water : 0.05% trifluoroacetic acid-acetonitrile = 57:43); FAB-MS (m/z): 1619 $[M+H]^+$.

The following Aerothricins 100, 112, 114 and 115 were prepared according to the method similar to that described in Example 13.

	FAB-MS	HPLC	Analytical condition
Compound name	m/z: $[MH^+]$	retention time (min.)	(column) (flow rate; ratio of eluent*)
Aerothricin 100	1730	14.8	(F)(10 ml/min.; 56/44)
Aerothricin 112	1647	11.8	(F)(10 ml/min.; 57/43)
Aerothricin 114	1759	23.1	(C)(10 ml/min.; 60/40)
Aerothricin 115	1633	19.6	(F)(10 ml/min.; 59/41)

* ratio of 0.05% trifluoroacetic acid-water : 0.05% trifluoroacetic acid-acetonitrile

Example 14

Preparation of Aerothricin 120

To a mixture of Aerothricin 3 (500 mg, 0.326 mmol) and triethylamine (682 µl, 4.89 mmol) in MeOH (10 ml) was added acrylonitrile (214 µl, 3.27 mmol) at room temperature. The mixture was stirred for 20 h at room temperature. After the solvent was evaporated *in vacuo*, the residue was dissolved in *n*-butanol and washed with diluted hydrochloric acid and water successively. The organic layer was evaporated *in vacuo*. The crude residue was purified by preparative reverse HPLC, the detailed condition of which is shown below. The appropriate fraction were combined, frozen and lyophilized to give 207 mg of Aerothricin 120 as a colorless amorphous solid.

HPLC(Rt) 27.5 min. (column F, flow rate: 10 ml/min., eluent: 0.05% trifluoroacetic acid-water : 0.05% trifluoroacetic acid-acetonitrile = 53:47); FAB-MS (m/z): 1586 $[M+H]^+$.

Example 15

Prepration of Aerothricin 113

To a mixture of Aerothricin 120 (100 mg, 0.063 mmol) in MeOH (5 ml) was added 10% palladium on charcoal (20 mg), and the reaction vessel was filled with hydrogen. After being stirred for 20 h at room temperature, the mixture was filtered through membrane filter (pore size: 0.2 μm) and the solvent was evaporated *in vacuo*. The crude residue was purified by preparative reverse HPLC, the detailed condition of which is shown below. The appropriate fraction were combined, frozen and lyophilized to give 87.2 mg of Aerothricin 113 as a colorless amorphous solid.

HPLC(Rt) 23.0 min. (column F, flow rate: 10ml/min., mobile phase: 0.05% trifluoroacetic acid-water : 0.05% trifluoroacetic acid-acetonitrile = 57:43) ; FAB-MS (m/z): 1590 $[M+H]^+$.

Aerothricin 129 was prepared according to the method similar to that described in Example 14 - 15 followed by removal Boc group of the ornitine residue with trifluoroacetic acid. The starting material, in this case, was the N^{δ}-Boc derivative of the (D)-ornitin moiety of Aerothricin 106 obtained in the process similar to Example 16.

	FAB-MS	HPLC	Analytical condition
Compound name	m/z: $[MH^+]$	retention time (min.)	(column) (flow rate; ratio of eluent*)
Aerothricin 129	1705	33.8	(F)(10 ml/min.; 62/38)

* ratio of 0.05% trifluoroacetic acid-water : 0.05% trifluoroacetic acid-acetonitrile

Example 16

Preparation of Aerothricin 14

To a solution of N-Boc-Sarcosine (123 mg, 0.65 mmol), WSC·HCl (240 mg, 1.25 mmol) and DMAP (150 mg, 1.23 mmol) in CH_3CN (10 ml) was added a solution of Aerothricin 3 (100 mg, 0.065 mmol) in CH_3OH (3 ml). The mixture was stirred at room temperature for 15 h and then concentrated *in vacuo*. The residue was dissolved in *n*-BuOH (10 ml) and washed with H_2O (5 ml x 2, adjusted pH 3~4 with 1 N HCl). The

n-BuOH layer was concentrated *in vacuo* and the residue was dissolved in TFA (5 ml) at 0°C. After the solution was stirred at room temperature for 1 h, TFA was evaporated *in vacuo*. The residue was purified by preparative reverse phase HPLC to give 40.8 mg (39% yield) of Aerothricin 14 as a white amorphous powder.

HPLC(Rt): 23.1 min. (column C, flow rate: 9 ml/min., 0.05% trifluoroacetic acid-water : 0.05% trifluoroacetic acid-acetonitrile = 60:40); FAB-MS (m/z): 1605[MH^+].

The following Aerothricins 15, 21, 26-29 and 101-107, 109, 110 , 118, 130 and 131 were prepared according to the method similar to that described in Example 16 using the corresponding acid as a building block.

	FAB-MS	HPLC	Analytical condition
Compound name	m/z: [MH^+]	retention time (min.)	(column) (flow rate; ratio of eluent*)
Aerothricin 15	1631	24.0	(C)(9 ml/min.; 57/43)
Aerothricin 21	1663	18.0	(C)(9 ml/min.; 60/40)
Aerothricin 26	1650	19.9	(C)(9 ml/min.; 50/50)
Aerothricin 27	1676	22.5	(C)(9 ml/min.; 55/45)
Aerothricin 28	1636	20.9	(C)(9 ml/min.; 50/50)
Aerothricin 29	1606	31.0	(C)(9 ml/min.; 60/40)
Aerothricin 101	1647	16.5	(F)(10 ml/min.; 56/44)
Aerothricin 102	1661	16.3	(F)(10 ml/min.; 56/44)
Aerothricin 103	1689	13.4	(F)(10 ml/min.; 54/46)
Aerothricin 104	1633	22.6	(F)(10 ml/min.; 58/42)
Aerothricin 105	1619	29.2	(F)(10 ml/min.; 52/38)
Aerothricin 106	1647	17.3	(F)(10 ml/min.; 56/44)
Aerothricin 107	1661	36.5	(F)(10 ml/min.; 60/40)
Aerothricin 109	1633	26.1	(F)(10 ml/min.; 58/42)
Aerothricin 110	1619	28.8	(F)(9 ml/min.; 58/42)
Aerothricin 118	1685	15.2	(F)(10 ml/min.; 51/49)
Aerothricin 130	1847	16.0	(F)(10 ml/min.; 63/37)
Aerothricin 131	1818	21.1	(F)(10 ml/min.; 63/37)

* ratio of 0.05% trifluoroacetic acid-water : 0.05% trifluoroacetic acid-acetonitrile

Example 17

Preparation of Aerothricin 74

A mixture of Aerothricin 66 (20 mg, 0.012 mmol), 3,5-dimethylpyrazole-1-carboxamidine nitrate (13 mg, 0.064 mmol) and triethylamine (18 ml, 0.13 mmol) in MeOH (1 ml) was stirred at room temperature for 15 h. After solvent was evaporated, the crude residue was purified by preparative reverse phase HPLC, the detailed condition of which is shown below. The appropriate fractions were combined, frozen and lyophilized to give 10.2 mg of Aerothricin 74 as a colorless amorphous solid.

HPLC(Rt) 21.2 min. (column C, flow rate: 9 ml/min., eluent: 0.05% trifluoroacetic acid-water : 0.05% trifluoroacetic acid-acetonitrile = 54:46); FAB-MS (m/z): 1645[MH^+].

The following Aerothricins 4 and 116 were prepared according to the method similar to that described in Example 17 using Aerothricin 3 and 111 as a starting material, respectively.

	FAB-MS	HPLC	Analytical condition
Compound name	m/z: [MH^+]	retention time (min.)	(column) (flow rate; ratio of eluent*)
Aerothricin 4	1576	7.6	(D)(1 ml/min.; 50/50)
Aerothricin 116	1703	14.9	(F)(10 ml/min.; 57/43)

* ratio of 0.05% trifluoroacetic acid-water : 0.05% trifluoroacetic acid-acetonitrile

Example 18

Preparation of Aerothricin 5

(a). To a solution of Compound A, obtained in Reference Example 3, (10 mg, 0.0061 mmol) and potassium carbonate (10 mg, 0.072 mmol) in DMF (1 ml) was added methyl iodide (8 µl, 0.129 mmol) at room temperature and the mixture was stirred for 43 h at room temperature. After the mixture was filtered by Celite-pad and the filtrate was evaporated *in vacuo*. The residue was dissolved in *n*-butanol and washed with diluted hydrochloric acid and water successively. The organic layer was evaporated *in vacuo*. The crude residue was used for the next step without further purification.

(b). A solution of the crude residue obtained above in TFA (1.0 ml) was stirred at 0°C for 30 min. TFA was evaporated *in vacuo*. The residue was purified by preparative reverse phase HPLC, the detailed condition of which is shown below. The appropriate fractions were combined, frozen and lyophilized to give 3.8 mg of Aerothricin 5 as a colorless amorphous solid.

HPLC(Rt): 14.5 min. (column B, flow rate: 4 ml/min., eluent: 0.05% trifluoroacetic acid-water : 0.05% trifluoroacetic acid-acetonitrile = 55:45); FAB-MS (m/z): 1547[MH^+].

The following Aerothricins 6-10 and 76 were prepared according to the method similar to that described in Example 18 using the corresponding alkylating agent.

	FAB-MS	HPLC	Analytical condition
Compound name	m/z: [MH^+]	retention time (min.)	(column) (flow rate; ratio of eluent*)
Aerothricin 6	1561	16.0	(A)(1 ml/min.; 55/45)
Aerothricin 7	1573	8.4	(A)(1 ml/min.; 50/50)
Aerothricin 8	1589	26.1	(B)(4.7 ml/min.; 58/42)
Aerothricin 9	1591	38.5	(B)(4 ml/min.; 60/40)
Aerothricin 10	1590	6.7	(A)(1 ml/min.; 53/47)
Aerothricin 76	1617	26.0	(C)(9 ml/min.; 53/47)

* ratio of 0.05% trifluoroacetic acid-water : 0.05% trifluoroacetic acid-acetonitrile

Example 19

Preparation of Aerothricin 24

(a). A cold mixture of Compound A, obtained in Reference Example 3, (100 mg), sodium iodide (29.5 mg, 0.197 mmol) and sodium hypochlorite solution (250 µl) in methanol (2 ml) was stirred at 0°C for 2 h. The reaction mixture was quenched with saturated aqueous sodium thiosulfate, acidified with 1 N HCl and extracted with *n*-butanol. The combined organic extracts were evaporated *in vacuo*. At this point, the starting material was still remained. To complete the iodination reaction, the same experimental procedure was repeated. After the same work up, the residue was purified by

preparative reverse phase HPLC to give the iodino derivative of the Compound A as colorless solid (54 mg, 50% yield).

(b). A mixture of the iodido derivative of Compound A obtained above (23.8 mg), methyl acrylate (16 µl), triethylamine (40 µl) and palladium acetate (2.1 mg) in acetonitrile (250 µl) and N,N-dimethylformamide (750 µl) was heated at 70°C for 28 h. The resulting mixture was passed through C-18 short column and the residue was treated with trifluoroacetic acid (1 ml) at 0°C for 1 h. The resulting mixture was evaporated *in vacuo*. Purification of the residue by preparative reverse phase HPLC gave Aerothricin 24 as colorless solid (8.8 mg, 40% yield).

HPLC(Rt): 86.3 min. (column F, flow rate: 9 ml/min., eluent: 0.05% trifluoroacetic acid-water : 0.05% trifluoroacetic acid-acetonitrile = 58:42); FAB-MS (m/z): 1617[MH^+].

Example 20

Preparation of Aerothricin 96

A mixture of the iodido derivative of the Compound A (30 mg), obtained in Example 19(a), potassium acetate (6.9 mg) and tetrakis(triphenylphoshine)palladium (4.6 mg) in degassed dimethysulfoxide (2 ml) was heated at 60°C for 20 h under carbon monooxide atmosphere. The resulting mixture was passed through C-18 reverse phase short column and the residue was treated with trifluoroacetic acid at 0°C for 1 h. The resulting mixture was evaporated under reduced pressure. Purification of the residue by preparative reverse phase HPLC gave Aerothricin 96 as colorless solid (2.3 mg, 8% yield).

HPLC(Rt): 23.2 min. (column F, flow rate: 10 ml/min., eluent: 0.05% trifluoroacetic acid-water : 0.05% trifluoroacetic acid-acetonitrile = 52.2:47.8); FAB-MS (m/z): 1677[MH^+].

Example 21

Preparation of Aerothricin 32

(a). A mixture of the Compound A, obtained in Reference Example 3, (20 mg) and (methoxycarbonylsulfamoyl)triethylammonium hydroxide (26.5 mg, 0.108 mmol) in acetonitrile (3 ml) was stirred at room temperature for 8 h. The reaction mixture was

acidified with 1 N HCl and was evaporated *in vacuo*. The residue was extracted with *n*-butanol and the extracts were evaporated *in vacuo*.

(b). The crude product was treated with trifluoroacetic acid at 0°C for 1 h. TFA was evaporated *in vacuo*. Purification of the residue by preparative reverse phase HPLC gave Aerothricin 32 as colorless solid (2.0 mg, 10% yield).

HPLC(Rt): 42.9 min. (column B, flow rate: 4 ml/min., eluent: 0.05% trifluoroacetic acid-water : 0.05% trifluoroacetic acid-acetonitrile = 55:45); FAB-MS (m/z): 1516[MH^+].

Example 22

Preparation of Aerothricin 31

(a). To a cold solution of the Compound A, obtained in Reference Example 3, (25.7 mg) in tetrahydrofurane (5 ml) was added borane-dimethylsulfide complex (25 ml) at -10 °C. After being stirred at -10°C for 5 h, the reaction mixture was quenched with 2 N HCl and was extracted with *n*-butanol. The combined extracts were evaporated *in vacuo*.

(b). The crude product was treated with trifluoroacetic acid at 0°C for 1 h. THF was evaporated under reduced pressure. Purification of the residue by preparative reverse phase HPLC gave Aerothricin 31 as colorless solid (3.7 mg, 15% yield).

HPLC(Rt): 25.1 min. (column B, flow rate: 4 ml/min., eluent: 0.05% trifluoroacetic acid-water : 0.05% trifluoroacetic acid-acetonitrile = 62:38); FAB-MS (m/z): 1519[MH^+].

Example 23

Preparation of Aerothricin 121

To a solution of Aerothricin 3 (50 mg) in DMF(1 ml) and triethylamine (0.025 ml) was added methyl iodide (0.010 ml). After being stirred for 16 h at room temperature, to the mixture was further added triethylamine (0.025 ml) and methyl iodide (0.05 ml) and stirred for 24 h at room temperature. LCMS analysis of the mixture indicated > 90% conversion to the desired compound. The solvent was purged with a stream of nitrogen

and the residue was purified by preparative reverse phase HPLC, the detailed condition of which is shown below. The appropriate fractions were combined, frozen and lyophilized to give 23 mg of Aerothricin 121, as a colorless amorphous solid.

HPLC(Rt): 20.5 min. (column B, flow rate: 4 ml/min., eluent: 0.05% trifluoroacetic acid-water : 0.05% trifluoroacetic acid-acetonitrile = 52:48); FAB-MS (m/z): 1576[M^+].

Example 24

Preparation of Aerothricin 122

To a solution of Aerothricin 3 (50 mg) in pyridine(1 ml) was added sulfur trioxide N,N-dimethylformamide complex(23 mg). After being stirred for 2 h at room temperature, the solvent was purged with a stream of dry nitrogen.

A solution of the crude residue obtained above in TFA (1 ml) was stirred at 0°C for 30 min. TFA was purged with a stream of dry nitrogen and the residue was purified by preparative reverse phase HPLC, the detailed condition of which is shown below. The pure fractions were combined, frozen and lyophilized to give 5 mg of Aerothricin 122, as a colorless amorphous solid.

HPLC(Rt): 24.6 min. (column F, flow rate: 10 ml/min., eluent: 0.05% trifluoroacetic acid-water : 0.05% trifluoroacetic acid-acetonitrile = 52:48); FAB-MS (m/z): 1613[MH^+].

Example 25

Preparation of Aerothricin 63

(a). To a stirred solution of N^{α}-Fmoc-N^{β}-Boc-(S)-2,3-diaminopropionic acid (343 mg, 0.80 mmol) in DMF (10 ml) were added BOP reagent (355 mg, 0.80 mmol), HOBT hydrate (124 mg, 0.81 mmol) and N,N-diisopropylethylamine (0.174 ml, 1.00 mmol). After the mixture was stirred for 1.5 h at room temperature, a solution of Aerothricin 3 (1.10 g, 0.67 mmol) and N,N-diisopropylethylamine (0.174 ml, 1.00mmol) in DMF (9.5 ml) was added to the mixture. After being stirred for additional 1 h at room temperature, the mixture was concentrated *in vacuo*.

(b). To a stirred solution of the residue obtained above in DMF (20 ml) was added piperidine-4-carboxylic acid polyamine resin (200-400 mesh),HL (1.50 mmol/g, 2.66 g),

and the reaction mixture was irradiated with ultrasonic sound for 6 h. The resin was removed by filtration through a Celite pad, washed with MeOH and the combined filtrate and washing were frozen and lyophilized to give 1.08 g of the crude derivative of Aerothricin 3 as a white amorphous solid, which was used for the next step without further purification.

(c). To a stirred solution of the crude derivative of Aerothricin 3, obtained above, (25.6 mg, 0.015 mmol) in MeOH (1 ml) were added (2-oxo-ethyl)carbamic acid *tert*-butyl ester (crude, 207 mg), AcOH (0.1 ml) and $NaBH_3CN$ (19.1 mg). After the mixture was stirred for 2 h at room temperature, the reaction mixture was concentrated *in vacuo*. The residue was diluted with *n*-BuOH (4 ml) and washed with H_2O (1 ml x 2, adjusted pH 3-4 with 0.1 N HCl). The *n*-BuOH layer was concentrated *in vacuo*. The crude residue was used for the next step without further purification.

(d). A solution of the crude residue obtained above in TFA (2 ml) was stirred at 0°C for 2 h. TFA was evaporated *in vacuo* and the residue was purified by preparative reverse phase HPLC, the detailed condition of which is shown below. The pure fractions were combined, frozen and lyophilized to give 8.8 mg of Aerothricin 63 as a white amorphous solid.

HPLC(Rt): 24.8 min. (column F, flow rate: 9 ml/min., eluent: 0.05% trifluoroacetic acid-water : 0.05% trifluoroacetic acid-acetonitrile = 54:36); FAB-MS(m/z): 1706 [MH^+].

Example 26

Preparation of Aerothricin 127

Aerothricin 127 was prepared by the same method as that described for Aerothricin 63 by use of N^{α}-Fmoc-N^{δ}-Boc-(D)-ornitin. Aerothricin 127 was obtained as a white amorphous solid.

HPLC(Rt): 23.9 min. (column F, flow rate: 9 ml/min., eluent: 0.05% trifluoroacetic acid-water: 0.05% trifluoroacetic acid-acetonitrile = 54:36); FAB-MS(m/z): 1734 [MH^+].

Example 27

Preparation of Aerothricin 124

(a). To a stirred solution of Boc-D-Orn(Boc)-OH (46 mg, 0.138 mmol) in DMF (2 ml) were added BOP reagent (62 mg, 0.14 mmol), HOBT hydrate (22 mg, 0.144 mmol) and N,N-diisopropylethylamine (24 µl, 0.138 mmol). After being stirred for 30 min. at room temperature, a solution of Aerothricin 120 (100 mg, 0.063 mmol) and N-diisopropylethylamine (24 µl, 0.138 mmol) in DMF (2 ml) was added to the reaction mixture. After being stirred for 18 h at room temperature, the solvent was evaporated *in vacuo*.

The residue was dissolved in TFA (4 ml), and the solution was stirred at 0°C for 30 min. After removal of TFA with a stream of dry nitrogen, the residue was purified by preparative reverse phase HPLC, the detailed condition of which is shown below. The pure fractions were combined, frozen and lyophilized to give 48.6 mg of the nitrile derivative as a white amorphous solid.

HPLC(Rt): 20.2 min. (column F, flow rate: 10 ml/min., eluent: 0.05% trifluoroacetic acid-water : 0.05% trifluoroacetic acid-acetonitrile = 57:43); FAB-MS (m/z): 1700 $[M+H]^+$.

(b). To a mixture of the nitrile derivative obtained above (48.6 mg, 0.0286 mmol) in dioxane (1 ml) and water (1 ml) was added 10% palladium on charcoal (10 mg), and the mixture was stirred under hydrogen atmosphere for 14 h at room temperature. Then the mixture was filtered through membrane filter (pore size: 0.2 µm) and the solvent was evaporated *in vacuo*. The crude residue was purified by preparative reverse phase HPLC, the detailed condition of which is shown below. The pure fractions were combined, frozen and lyophilized to give 26.5 mg of Aerothricin 124 as a colorless amorphous solid.

HPLC(Rt): 18.2 min. (column F, flow rate: 10 ml/min., eluent: 0.05% trifluoroacetic acid-water : 0.05% trifluoroacetic acid-acetonitrile = 60:40); FAB-MS (m/z): 1704 $[M+H]^+$.

Example 28

Preparation of Aerothricin 125

(a). To a solution of Aerothricin 3 mono TFA salt (natural product: 50 mg) in DMF(1 ml) and triethylamine(0.126 ml) was added 2-bromo-5-nitropyridine(185 mg). After being stirred for 25 h at room temperature, the solvent was purged with a stream of dry nitrogen. The residue was purified by preparative reverse phase HPLC. The appropriate fractions were combined, frozen and lyophilized to give 25 mg of 5-nitropyrid-2-yl derivative of Aerothricin 3 as a slight yellow amorphous solid.

HPLC(Rt): 29.9 min. (column F, flow rate: 10 ml/min., eluent: 0.05% trifluoroacetic acid-water : 0.05% trifluoroacetic acid-acetonitrile = 47:53); FAB-MS (m/z): 1655 $[M+H]^+$.

(b). 5-Nitropyrid-2-yl derivative of Aerothricin 3 obtained above (10 mg) was dissolved in dioxane-H_2O (1 ml-5 ml). 5% Palladium on charcoal (20 mg) was added and the reaction vessel was filled with hydrogen. After being stirred for 3 h at room temperature, filtration through membrane filter (pore size: 0.2 μm) and evaporation of solvent gave 14 mg of crude product, which was purified by preparative reverse phase HPLC, the detailed condition of which is shown below. The pure fractions were combined, frozen and lyophilized to give 2.5 mg of Aerothricin 125 as a colorless amorphous solid.

HPLC(Rt): 18.7 min. (column F, flow rate: 10 ml/min., eluent: 0.05% trifluoroacetic acid-water : 0.05% trifluoroacetic acid-acetonitrile = 52:48); FAB-MS (m/z): 1625 $[M+H]^+$.

Example 29

Preparation of Aerothricin 128

(a). To a stirred solution of Fmoc-D-Orn(Boc)-OH (389 mg, 0.86 mmol) in DMF (10 ml) were added BOP reagent (378 mg, 0.85 mmol), HOBT hydrate (131 mg, 0.86 mmol) and N,N-diisopropylethylamine (171 μl, 0.98 mmol). After the mixture was stirred at a room temperature for 40 min., a solution of Aerothricin 3 (1.08 g, 0.66 mmol) and N,N-diisopropylethylamine (171 μl, 0.98 mmol) in DMF (10 ml) was added to the mixture. After being stirred for 2.5 h at a room temperature, piperidine (4 ml) was added, and the mixture was stirred for additional 1 h at a room temperature. The mixture was

concentrated *in vacuo*. The residue was diluted with *n*-BuOH (50 ml) and washed with H_2O (25 ml x 2, adjusted pH 3 with 1 N HCl). The *n*-BuOH layer was concentrated *in vacuo*.

(b). To a stirred solution of Boc-D-Orn(Boc)-OH (9.6 mg, 0.029 mmol) in DMF (1ml) were added BOP reagent (13.3 mg, 0.030 mmol), HOBT hydrate (4.6 mg, 0.030 mmol) and N,N-diisopropylethylamine (4.8 µl, 0.028 mmol). After the mixture was stirred at room temperature for 30 min., a solution of the crude residue (31.9 mg) obtained above and N,N-diisopropylethylamine (4.8 µl, 0.028 mmol) in DMF (1 ml) was added to the mixture. After being stirred for 4 h at a room temperature, the reaction mixture was concentrated *in vacuo*.

(c). The crude residue obtained above was dissolved in TFA (1.5 ml) and stirred at 0°C for 1 h. The reaction mixture was concentrated *in vacuo*, and the residue was purified by preparative reverse HPLC: The appropriate fraction were combined, frozen and lyophilized to give 16.6 mg of Aerothricin 128 as a white amorphous solid:

HPLC(Rt): 27.23 min. (column F, flow rate: 9 ml/min., eluent: 0.05% trifluoroacetic acid-water : 0.05% trifluoroacetic acid-acetonitrile = 55:35); FAB-MS (m/z): 1762 [MH^+].

Example 30

Preparation of Aerothricin 106 from the Compound (IX)

(a). A mixture of Fmoc-Tyr(Bu^t) (21 mg, 0.0457 mmol), HOBt mono hydrate (6.6 mg, 0.0431 mmol), BOP reagent (18.8 mg, 0.0424 mmol) and diisopropylethylamine (DIEA , 20µl) in DMF (0.5 ml) was stirred at room temperature for 1 h and then was added to a mixture of N(*orn*)-Boc-IX (19.3 mg, 0.0131 mmol) obtained in Example 6 and DIEA (10 µl) in DMF (1 ml). After stirring at room temperature for 3 h, the resulting mixture was treated with piperidine (0.375 ml) for 1 h and then was concentrated *in vacuo*. The residue was washed with dichloromethane and diethylether to remove the reagents. Purification of the residue by HPLC gave the desired linear peptide A as a white solid (16.6 mg).

HPLC (Rt) 19 min. (column: Soken-ODS / 20 x 250 mm, flow rate: 9 ml/min., eluent H_2O : CH_3CN = gradient, 1% AcOH).

(b). A mixture of Fmoc-D-Ala mono hydrate (1.2 mg, 0.034 mmol), HOBt mono hydrate (4.7 mg, 0.031 mmol), BOP reagent (13.6 mg, 0.031 mmol) and DIEA (8 µl) in DMF (0.5 ml) was stirred at room temperature for 1 h and then was added to a mixture of the linear peptide A obtained above (16.6 mg, 0.0098 mmol) and DIEA (6 µl) in DMF (1 ml). The reaction mixture was stirred at room temperature and the activated ester was added until the almost starting material was consumed. The resulting mixture was concentrated *in vacuo*. The residue was washed with dichloromethane and diethylether to remove the reagents. The crude product was treated with trifluoroacetic acid at 0°C for 1h. The mixture was concentrated under reduced pressure. Purification of the residue by HPLC gave the desired linear peptide B as a white solid (6.1 mg).

HPLC(Rt) 19 min. (column: Soken-ODS / 20 x 250 mm, flow rate: 9 ml/min., eluent: H_2O : CH_3CN = gradient, 1% AcOH).

(c). A mixture of Boc-D-Orn(Bu^t) (5.7 mg, 0.017 mmol), HOBt mono hydrate (2.3 mg, 0.015 mmol), BOP reagent (5.4 mg, 0.012 mmol) and DIEA (6 µl,) in DMF (0.5 ml) was stirred at room temperature for 1 h and then was added to a mixture of the linear peptide B (6.1 mg, 0.0033 mmol) and DIEA (3 µl) in DMF (1 ml). After stirring at room temperature for 2 h, the resulting mixture was treated with piperidine (0.375 ml) for 1 h. and was concentrated *in vacuo*. Purification of the residue by HPLC gave linear peptide C as a white solid (4.1 mg).

HPLC(Rt) 16.7 min. (column: Soken-ODS / 20 x 250 mm, flow rate: 9 ml/min., eluent H_2O : CH_3CN = gradient, 1% AcOH).

(d). The linear peptide C was acidified with 0.01 N hydrochloride and was extracted with *n*-butanol. The butanol extract was concentrated *in vacuo*. The extract was dissolved into DMF (2 ml). Then HOBt mono hydrate (0.1M in DMF, 60 µl), BOP reagent (0.1 M in DMF, 60 µl) and DIEA (2 µl) were added to the mixture. After stirring at room temperature for 1 h, the resulting mixture was concentrated *in vacuo*. The residue was treated with trifluoroacetic acid at 0°C for 1 h. The mixture was concentrated under reduced pressure. Purification of the residue by HPLC gave Aerothricin 106 as a white solid (2.2 mg, 9% from N(*orn*)-Boc-IX).

The analytical data is described in the table of Example 16.

Example A

Injectable solutions each containing the following ingredients were manufactured in the conventional manner per se:

Aerothricin 45	20 mg
di-Sodium hydrogenphosphate, anhydrous	7.6 mg
Sodium diphosphate dihydrate	2.0 mg
Ethyl alcohol	150 mg
Distilled water, deionized, sterile	850 mg
Total	1029.6 mg

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

DEPOSITOR
Name: Mr. Hiroaki Shigeta
President, Representative Director,
Nippon Roche K.K.

Address: 6-1, Shiba 2-chome, Minato-ku,
Tokyo, 105 Japan

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: Deuteromycotina NR7379	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: FERM BP-6391

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I above was accompanied by

(X) a scientific description
(X) a proposed taxonomic designation

(Mark with a cross where applicable).

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I. above, which was received by it on June 16, 1998(Date of the original deposit).

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion)

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: National Institute of Bioscience and Human-Technology Agency
of Industrial Science and Technology

Shinichi Ohashi, Dr. DIRECTOR GENERAL

Address: 1-3, Higashi 1-chome Tsukuba-shi Ibaraki-ken 305-8566, JAPAN

Date: June 16, 1998

[特許手続上の微生物の寄託の国際的承認に関するブダペスト条約]

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

下記国際寄託当局によって規則7．1に従い発行される。

issued pursuant to Rule 7.1 by the INTERNATIONAL DEPOSITARY AUTHORITY identified at the bottom of this page.

原寄託についての受託証

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT

寄託者、 氏名（名称） 日本ロシュ株式会社
代表取締役社長 栗田 寛昭 殿

あて名 〒
東京都港区芝二丁目6番1号

1．微生物の表示

（寄託者が付した識別のための表示）	（受託番号）
Deuteromycotina NR7379	FERM BP- 6391

2．科学的性質及び分類学上の位置

1種の微生物には、次の事項を記載した文書が添付されていた。

- ■ 科学的性質
- ■ 分類学上の位置

3．受領及び受託

本国際寄託当局は、平成10年 6月16日（原寄託日）に受領した1種の微生物を受託する。

4．移管請求の受領

本国際寄託当局は、 年 月 日（原寄託日）に1種の微生物を受領した。
そして、 年 月 日に原寄託よりブダペスト条約に基づく寄託への移管請求を受領した。

5．国際寄託当局

通商産業省工業技術院生命工学工業技術研究所

名称： National Institute of Bioscience and Human-Technology Agency of Industrial Science and Technology

所長 大島 泰郎 Dr. Shujiro Ohshima, Director-General

あて名： 日本国茨城県つくば市東1丁目1番3号（郵便番号305－8566）
1-3, Higashi 1 chome Tsukuba-shi Ibaraki-ken 305-8566, JAPAN

平成10年（1998） 6月16日

AUTHORIZATION BY THE DEPOSITOR UNDER RULE 28(1)(d)

The undersigned,

Hiroaki Shigeta, President and Representative Director of Nippon Roche K.K.,

confirms that

Nippon Roche K.K., of 6-1, Shiba 2-chome, Minato-ku, Tokyo, 105 Japan

has deposited on June 16, 1998 with

the National Institute of Bioscience and Human-Technology,
Agency of Industrial Science and Technology,
1-3, Higashi 1-chome, Tsukuba-shi,
Ibaraki-ken, 305 Japan

under accession number Deuteromycotina NR7379 (FERM BP-6391) biological material in accordance with the Budapest Treaty.

The undersigned DEPOSITOR hereby authorizes

F. Hoffmann-La Roche AG, Grenzacherstrasse 124,
CH-4070 Basel, SCHWEIZ
Name of Applicant

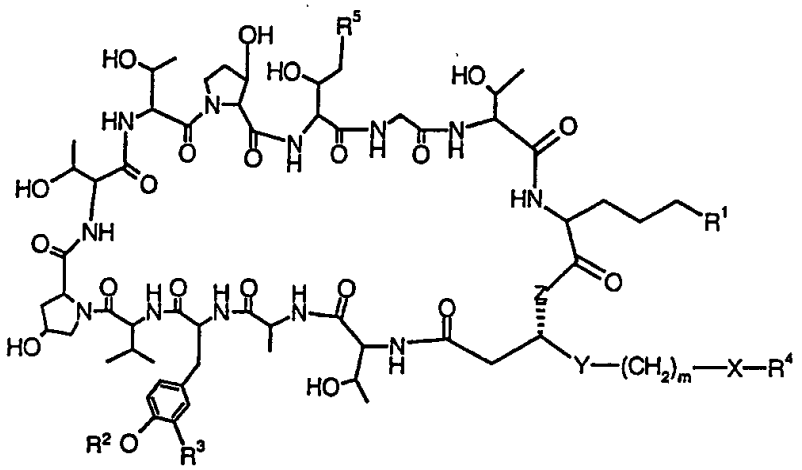
to refer to the aforementioned deposited biological material in the European Patent Application No. 98113744.1 and gives his unreserved and irrevocable consent to the deposited material being made available to the public in accordance with Rule 28 EPC.

signed: _____ dated: March 26, 1999

(Hiroaki Shigeta, President and Representative Director of Nippon Roche K.K.)

Claims

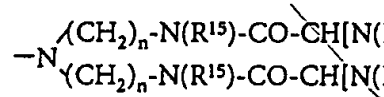
1. Aerothricins represented by the Formula (I),

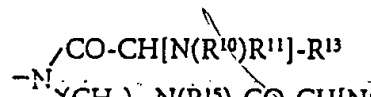


(I)

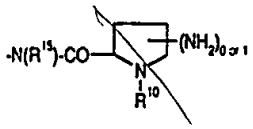
wherein

R^1 is guanidino, tri-lower alkylammonio, $-N(R^{10})-R^{11}$, $-N(R^{15})-CO-R^{14}$, $-N(R^{15})-CO-CH[N(R^{10})R^{11}]-R^{13}$, $-NHCOCH(R^{13})-NHCOCH(NH_2)-R^{13}$,





, or



;

R^{10} and R^{11} are each independently selected from hydrogen; heteroaryl substituted with one or two amino; lower alkyl optionally substituted with one or more amino, amino-lower alkyl, cyano, guanidino, nitrogen containing heterocycle(s) or phenyl group(s) containing an amino, amidino or guanidino group;

R^{13} is a residue derived from natural or unnatural amino acids;

R^{14} is lower alkyl substituted with one or more amino, guanidino, nitrogen containing heterocycle(s) or phenyl group(s) containing an amino, amidino or guanidino group;

R^{15} is hydrogen, lower alkyl optionally substituted with one or more amino, guanidino, nitrogen containing heterocycle(s) or phenyl group(s) containing an amino, amidino or guanidino group;

R^2 is hydrogen, hydroxysulfonyl, lower alkyl or lower alkenyl, wherein lower alkyl and lower alkenyl may be optionally substituted with acyl, carbamoyl, amino, mono-lower alkylamino or di-lower alkylamino;

R^3 is hydrogen, hydroxy, nitro, amino, acylamino, (lower alkylcarbamoyl)amino, carboxyl, lower alkoxy, lower alkoxycarbonyl, lower alkyl, lower alkenyl or lower alkynyl, wherein lower alkyl, lower alkenyl and lower alkynyl may be optionally substituted with hydroxy, amino, mono-lower alkylamino, di-lower alkylamino, lower alkoxycarbonyl or carbamoyl;

R^4 is alkyl, alkenyl, alkoxy or alkenyloxy which may be optionally substituted with lower alkyl, aryl, cycloalkyl or fluorine atom(s);

R^5 is $-CONH_2$, -CN or $-CH_2NH_2$;

X is a single bond, or an aryl, biphenyl or terphenyl group optionally containing one or more hetero atom(s) and/or being substituted with halogen atom(s) or lower alkyl;

Y is a single bond, $-CH_2-$, -CH(lower alkyl)-, -CONH- or -CON(lower alkyl)-;

Z is -O-, -NH- or -N(lower alkyl)-;

m is an integer of 0 to 4; and

n is an integer of 2 to 5;

with the proviso that when $-Y-(CH_2)m-X-R^4$ is unsubstituted alkyl or aralkyl, then R^1 is not amino, R^2 and R^3 are not hydrogen, R^5 is not $-CONH_2$, and Z is not -O- or -NH- at the same time;

and pharmaceutically acceptable salts thereof.

2. Aerothricins of Claim 1 wherein R^1 is $-N(R^{10})-R^{11}$, with R^{10} and R^{11} as defined in Claim 1.

3. Aerothricins of Claim 1 wherein R^1 is -N(R^{15})-CO-CH[N(R^{10})R^{11}]-R^{13}, with R^{10}, R^{11}, R^{13} and R^{15} as defined in Claim 1.

4. Aerothricins of Claim 1 wherein R^1 is -NHCOCH(R^{13})-NHCOCH(NH_2)-R^{13}, with R^{13} as defined in Claim 1.

5. Aerothricins of Claim 1 wherein R^1 is

$$-N\begin{matrix}\nearrow (CH_2)_n\text{-}N(R^{15})\text{-}CO\text{-}CH[N(R^{10})R^{11}]\text{-}R^{13} \\ \searrow (CH_2)_n\text{-}N(R^{15})\text{-}CO\text{-}CH[N(R^{10})R^{11}]\text{-}R^{13},\end{matrix}$$

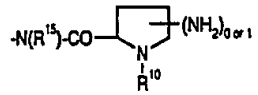
with R^{10}, R^{11},R^{13}, R^{15} and n as defined in Claim 1.

6. Aerothricins of Claim 1 wherein R^1 is

$$-N\begin{matrix}\nearrow CO\text{-}CH[N(R^{10})R^{11}]\text{-}R^{13} \\ \searrow (CH_2)_n\text{-}N(R^{15})\text{-}CO\text{-}CH[N(R^{10})R^{11}]\text{-}R^{13},\end{matrix}$$

with R^{10}, R^{11},R^{13}, R^{15} and n as defined in Claim 1.

7. Aerothricins of Claim 1 wherein R^1 is -N(R^{15})-CO-R^{14}, with R^{14} and R^{15} as defined in Claim 1.

8. Aerothricins of Claim 1 wherein R^1 is  , with R^{10} and R^{15} as defined in Claim 1.

9. Aerothricins of Claim 1 wherein R^1 is amino or guanidino.

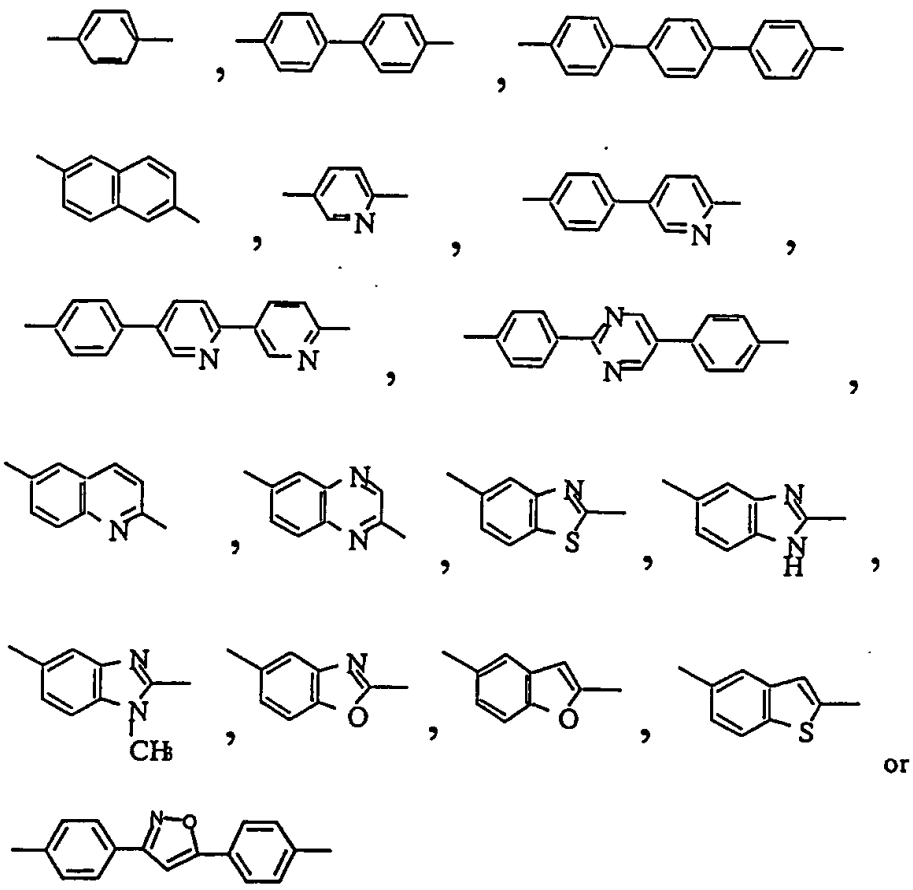
10. Aerothricins of any of Claims 1 to 9 wherein R^2 is hydrogen, hydroxysulfonyl or lower alkyl.

11. Aerothricins of any of Claims 1 to 10 wherein R^3 is hydrogen, hydroxy, nitro, amino or acylamino.

12. Aerothricins of any of Claims 1 to 10 wherein R^3 is (lower alkylcarbamoyl)amino, carboxyl, lower alkoxy or lower alkoxycarbonyl.

13. Aerothricins of any of Claims 1 to 12 wherein R^5 is -$CONH_2$ or -CH_2NH_2.

14. Aerothricins of any of Claims 1 to 13 wherein X is a single bond or one of the following radicals:



which may be further substituted with halogen atom(s) or lower alkyl.

15. Aerothricins of any of Claims 1 to 13 wherein X is a single bond, phenyl, biphenyl or naphtyl which may be further substituted with halogen atom(s) or lower alkyl.

16. Aerothricins of any of Claims 1 to 15 wherein R^4 is alkyl or alkoxy which may be optionally substituted with lower alkyl, aryl, cycloalkyl or fluorine atom(s).

17. Aerothricins of any of Claims 1 to 16 wherein m is an integer of 0 to 2.

18. Aerothricins of any of Claims 1 to 17 wherein Y is $-CH(CH_3)-$, $-CON(CH_3)-$, $-CONH-$, $-CH_2-$ or a single bond.

19. Aerothricins of any of Claims 1 to 18 wherein Z is -NH-.

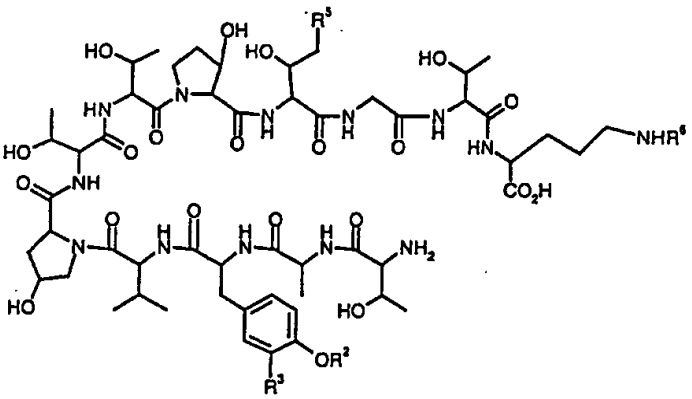
20. Aerothricins of any of Claims 1 to 18 wherein Z is -O-.

21. Aerothricins of any of Claims 1 to 20 selected from the group consisting of Aerothricins 2, 4 to 32, 63, 96-99, 101 to 131.

22. Aerothricins of any of Claims 1 to 20 selected from the group consisting of Aerothricins 14, 15, 21, 26 to 29, 63, 98, 99 and 101 to 131.

23. Aerothricins of any of Claims 1 to 22 for the use in medical therapy.

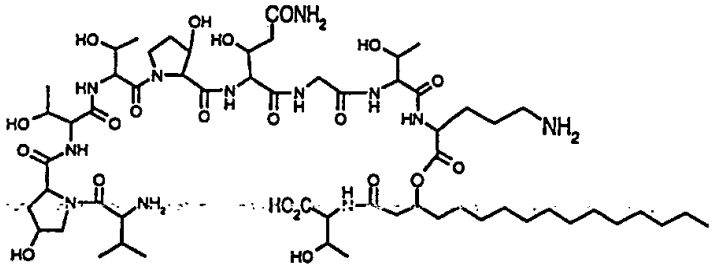
24. Compounds represented by the Formula (III)



(III)

wherein R^2, R^3 and R^5 are as defined in Claim 1 and R^6 is an amino protecting group; with the proviso that when R^5 is $-CONH_2$, then R^2 or R^3 are other than hydrogen; and salts thereof.

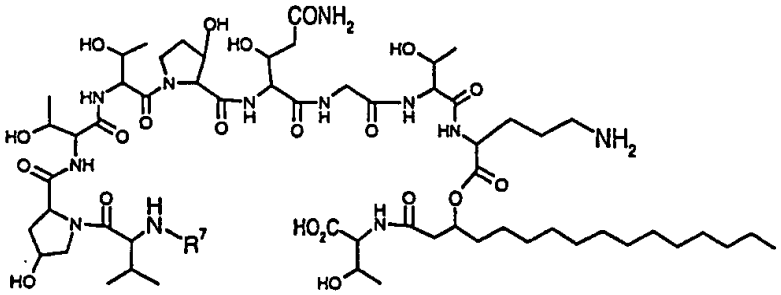
25. A compound represented by the Formula (IX),



(IX)

and salts thereof.

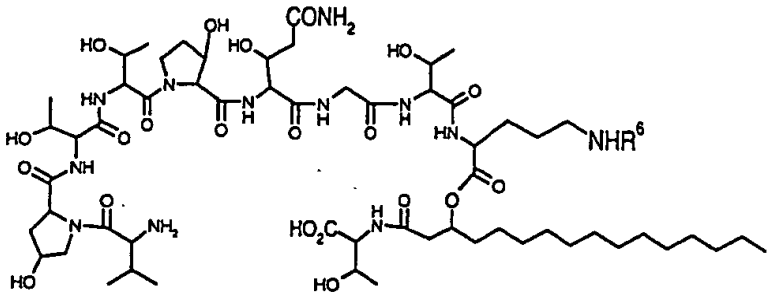
26. A compounds represented by the Formula (X),



(X)

wherein R^7 is an amino protecting group, and salts thereof.

27. Compounds represented by the Formula (XII),



(XII)

wherein R^6 is an amino protecting group, and salts thereof.

28. A pharmaceutical composition comprising a compound of any of Claims 1 to 22 and a pharmaceutically acceptable carrier.

29. The use of a compound as defined in any of Claims 1 to 22 for the preparation of medicaments for the treatment or prophylaxis of mycoses.

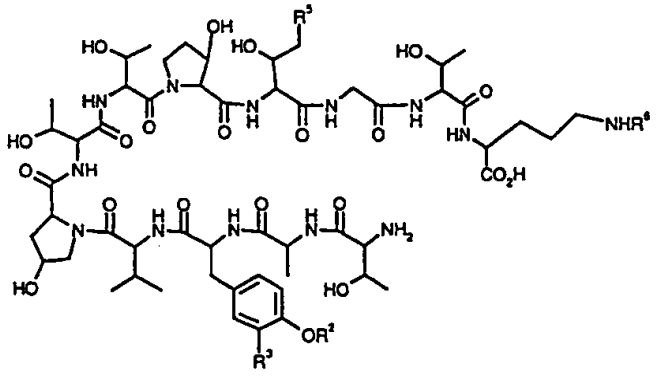
30. A biologically pure culture of Deuteromycotina NR7379 (FERM BP-6391).

31. A method for the prophylactic and/or therapeutic treatment of infectious diseases caused by pathogenic microorganisms which comprises administering a compound of any of Claims 1 to 22 to a human being or an animal.

32. A process for the preparation of the Aerothricins described in any of Claims 1 to 22, which process comprises:

(a) cultivating a microorganism belonging to the genus *Deuteromycotina* and isolating Aerothricin 1, 2 and/or a linear peptide of the Formula (IX) as defined in Claim 25 from the culture broth; or

(b) condensation of a compound of the Formula (III),



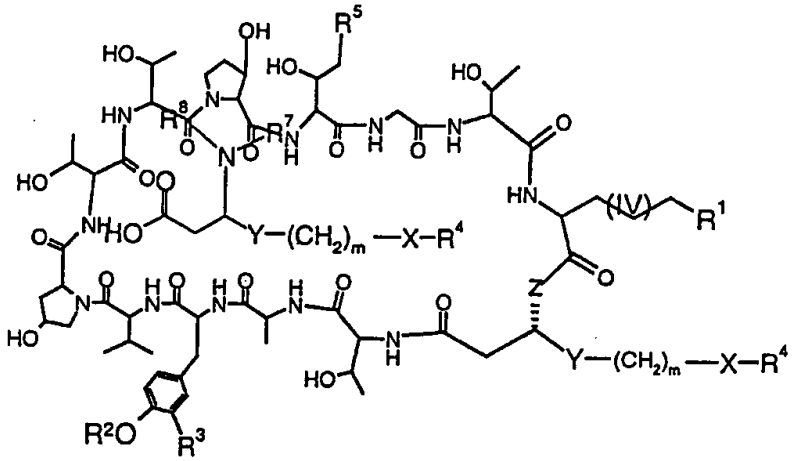
(III)

wherein R^2, R^3 and R^5 are as defined in Claim 1 and R^6 is an amino protecting group; with a compound of the Formula (IV),

wherein

R^7 is an amino protecting group, R^8 is hydrogen or lower alkyl; and R^4, X, Y and m are as defined in Claim 1; followed by removal of the amino protecting group R^7, the successive cyclization and removal of the amino protecting group R^6, or

(c) nitration of Aerothricins of the Formula (I),



(I)

wherein R^3 is a hydrogen atom; and R^1, R^2, R^4, R^5, X, Y, Z and m are as defined in Claim 1; or

(d) reduction of the nitro group of Aerothricins of the above Formula (I), wherein R^3 is a nitro group; and R^1, R^2, R^4, R^5, X, Y, Z and m are as defined in Claim 1; or

(e) acylation of the amino group of Aerothricins of the above Formula (I), wherein R^3 is an amino group; and R^1, R^2, R^4, R^5, X, Y, Z and m are as defined in Claim 1; followed, if necessary, by removal of the amino protecting group, or

(f) cyanoethylation or 2,2-dicyanoethylenation of the amino group of Aerothricins of the Formula (I), wherein R^1 is an amino group or $-N(R^{15})-COCH(NH_2)-R^{13}$; and R^{13}, R^{15}, R^2, R^3, R^4, R^5, X, Y, Z and m are as defined in Claim 1; followed by reduction of the cyano group(s), and if necessary by removal of protecting group(s), or

(g) reductive alkylation of the amino group of Aerothricins of the above Formula (I), wherein R^1 is amino, (2-cyanoethyl)amino or $-N(R^{15})-CO-CH[N(R^{10})R^{11}]-R^{13}$; with R^{10} and R^{11} each independently selected from hydrogen or (2-cyanoethyl)amino; and R^{13}, R^{15}, R^2, R^3, R^4, R^5, X, Y, Z and m as defined above; with an aldehyde of the formula (V),

R^9-CHO (V)

wherein R^9 is hydrogen, lower alkyl which may be further substituted with one or more protected amino, nitrogen containing heterocycle(s) or phenyl group(s) containing a protected amino group; followed, if necessary, by removal of amino protecting group(s) or reduction of a cyano group, or

(h) arylation of the amino group of Aerothricins of the above Formula (I), wherein R^1 is an amino group; and R^2, R^3, R^4, R^5, X, Y, Z and m are as defined in Claim 1; with a compound of the formula (VI),

R^{12}-Q (VI)

wherein R^{12} is a nitrogen containing heteroaryl which may be further substituted with a protected amino or nitro group and Q is a halogen atom such as chloro or bromo; followed, if necessary, by removal of amino protecting group or reduction of a nitro group, or

(i-1) acylation of the amino group of Aerothricins of the above Formula (I), wherein R^1 is amino and R^2, R^3, R^4, R^5, X, Y, Z and m are as defined in Claim 1; followed, if necessary, by removal of the amino protecting group, or

(i-2) acylation of the amino group of Aerothricins of the above Formula (I), wherein R^1 is -N(R^{10})-R^{11} [wherein R^{10} and R^{11} are both lower alkyl substituted with an amino group] or -N(R^{15})-CO-CH[N(R^{10})R^{11}]-R^{13} [wherein R^{15} is lower alkyl substituted with an amino group; R^{10}, R^{11}, and R^{13} are as defined in Claim 1 with the proviso that the amino group(s) present in R^{10}, R^{11} and R^{13} are protected], followed by removal of the amino protecting group, or

(j) mono N-alkylation of Aerothricins of the above Formula (I), wherein R^1 is amino; and R^2, R^3, R^4, R^5, X, Y, Z and m are as defined in Claim 1; and successive N-acylation, followed, if necessary, by removal of the amino protecting group, or

(k) conversion of the amino group of Aerothricins of the above Formula (I), wherein R^1 is an amino group; -N(R^{10})-R^{11} [wherein R^{10} and R^{11} are each independently selected from lower-alkyl optionally substituted with one or more amino or phenyl group(s) containing an amino group]; -N(R^{15})-CO-CH[N(R^{10})R^{11}]-R^{13} [wherein R^{10}, R^{11} and R^{13} are as defined in Claim 1 and R^{15} is lower alkyl optionally substituted with one or more amino group(s), nitrogen containing heterocycle(s) or phenyl group(s) containing an amino group]; or -NHCO-R^{14} [wherein R^{14} is lower-alkyl substituted with one or more amino, nitrogen containing heterocycle(s) or phenyl group(s) containing an amino group]

and R^2, R^3, R^4, R^5, X, Y, Z and m are as defined in Claim 1; into a guanidino group by treatment with an activated amidine derivative, or

(l) O-alkylation of the phenolic hydroxy group of Aerothricins of the above Formula (I), wherein R^2 is hydrogen and R^1, R^3, R^4, R^5, X, Y, Z and m are as defined in Claim 1; or

(m) iodination of Aerothricins of the above Formula (I), wherein R^2 and R^3 are hydrogen and R^1, R^4, R^5, X, Y, Z and m are as defined in Claim 1, followed by palladium(0) catalyzed coupling of the resulting iodo derivative of the Formula (I), wherein R^3 is an iodo and R^1, R^2, R^4, R^5, X, Y, Z and m are as defined in claim 1 and, if necessary, removal of the amino protecting group, or

(n) dehydration of the carbamoyl group of Aerothricins of the above Formula (I), wherein R^5 is $-CONH_2$ and R^1, R^2, R^3, R^4, X, Y, Z and m are as defined in Claim 1; followed, if necessary, by removal of the amino protecting group, or

(o) reduction of the carbamoyl or cyano group of Aerothricins of the above Formula (I), wherein R^5 is $-CONH_2$ or -CN and R^1, R^2, R^3, R^4, X, Y, Z and m are as defined in Claim 1; followed, if necessary, by removal of the amino protecting group, or

(p) hydroxy sulfonation of the tyrosine residue of Aerothricins of the above Formula (I), wherein R^2 is hydrogen and R^1, R^3, R^4, R^5, X, Y, Z and m are as defined in Claim 1, followed by removal of protecting group(s), or

(q) conversion of the linear peptide of the Formula above (IX) into Aerothricins of the above Formula (I) by peptide synthesis, followed by a modification according to a method selected from the above processes (c) to (o).

33. The invention as described hereinbefore.

Fig. 1

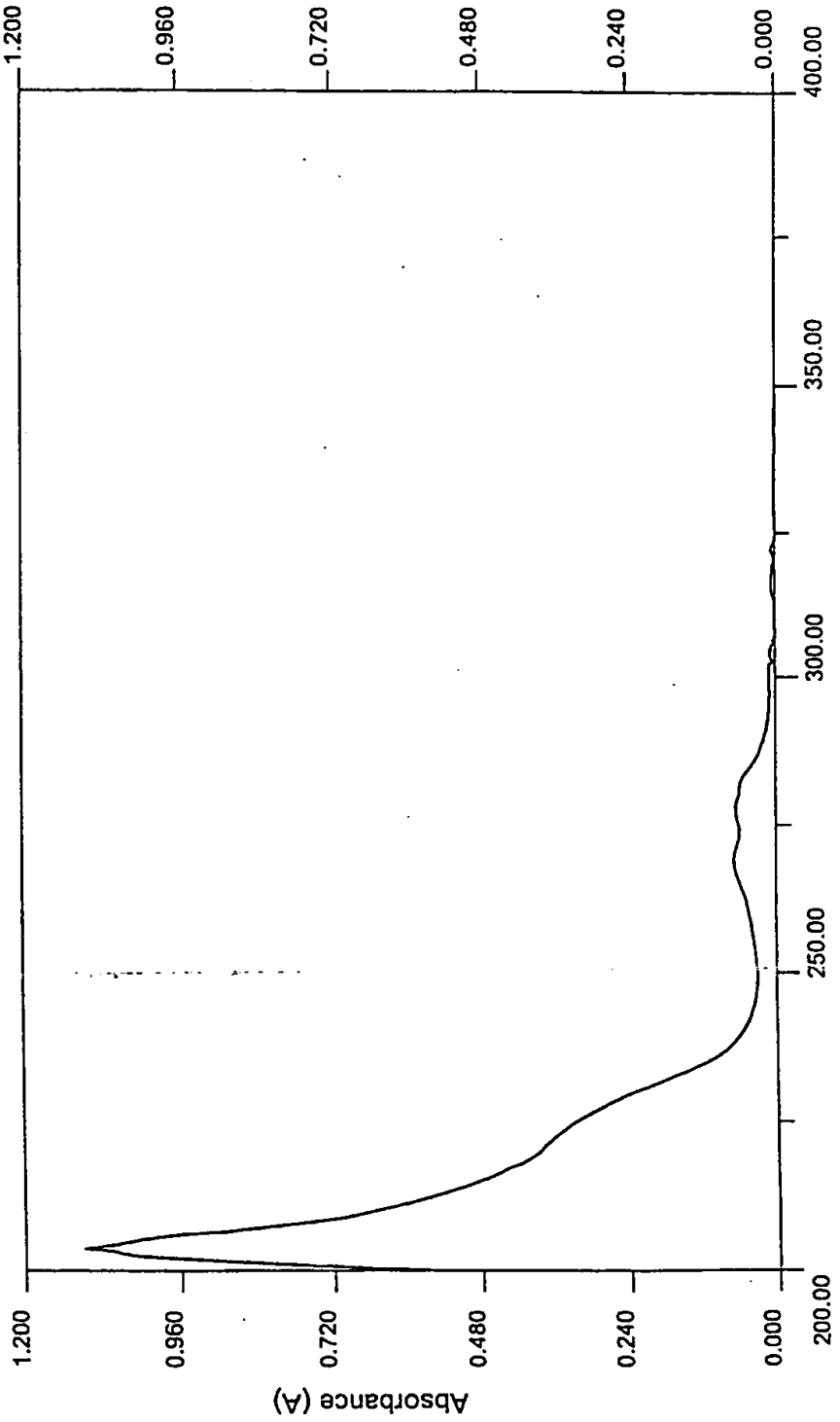

1.200
0.960
0.720
0.480
0.240
0.000
400.00
350.00
300.00
250.00
200.00
Absorbance (A)

Fig. 2

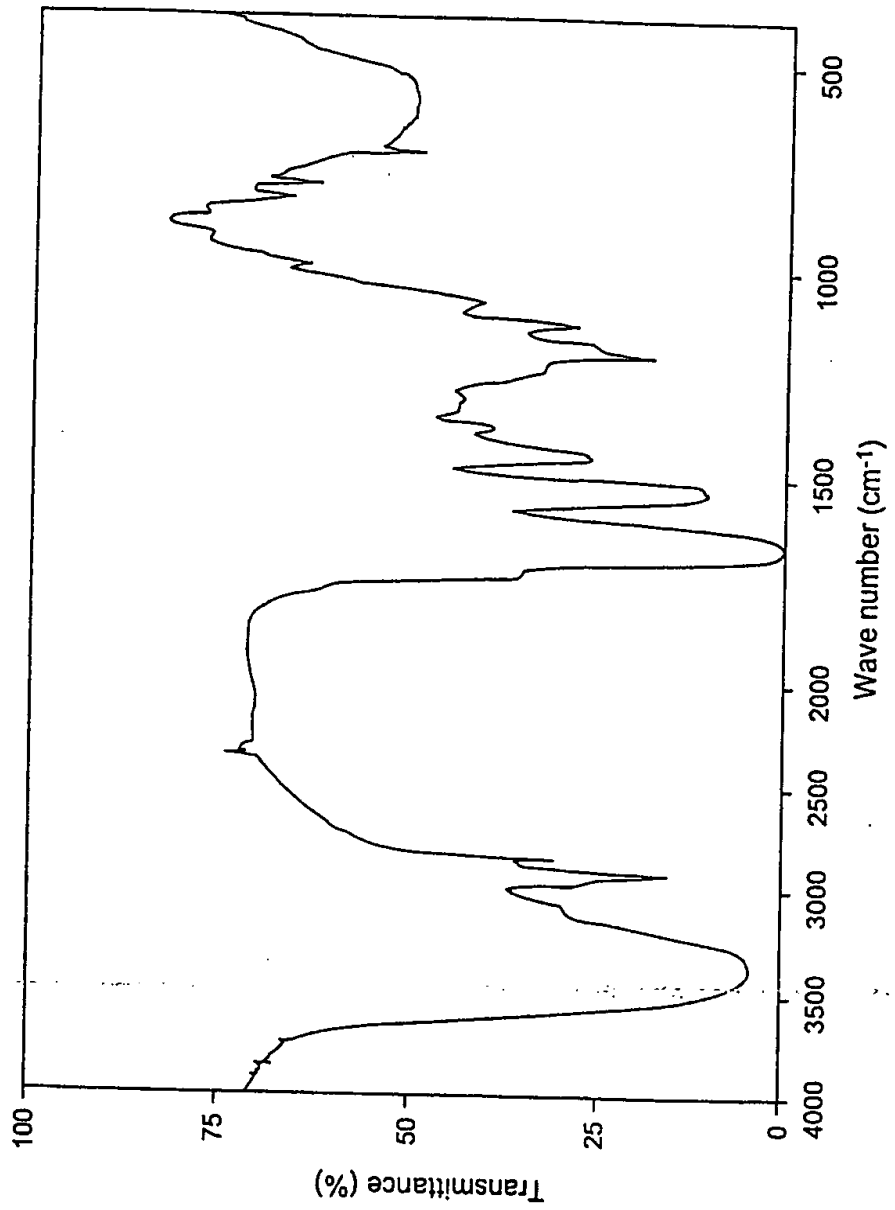

Transmittance (%)
100
75
50
25
0
4000
3500
3000
2500
2000
1500
1000
500
Wave number (cm-1)

Fig. 3

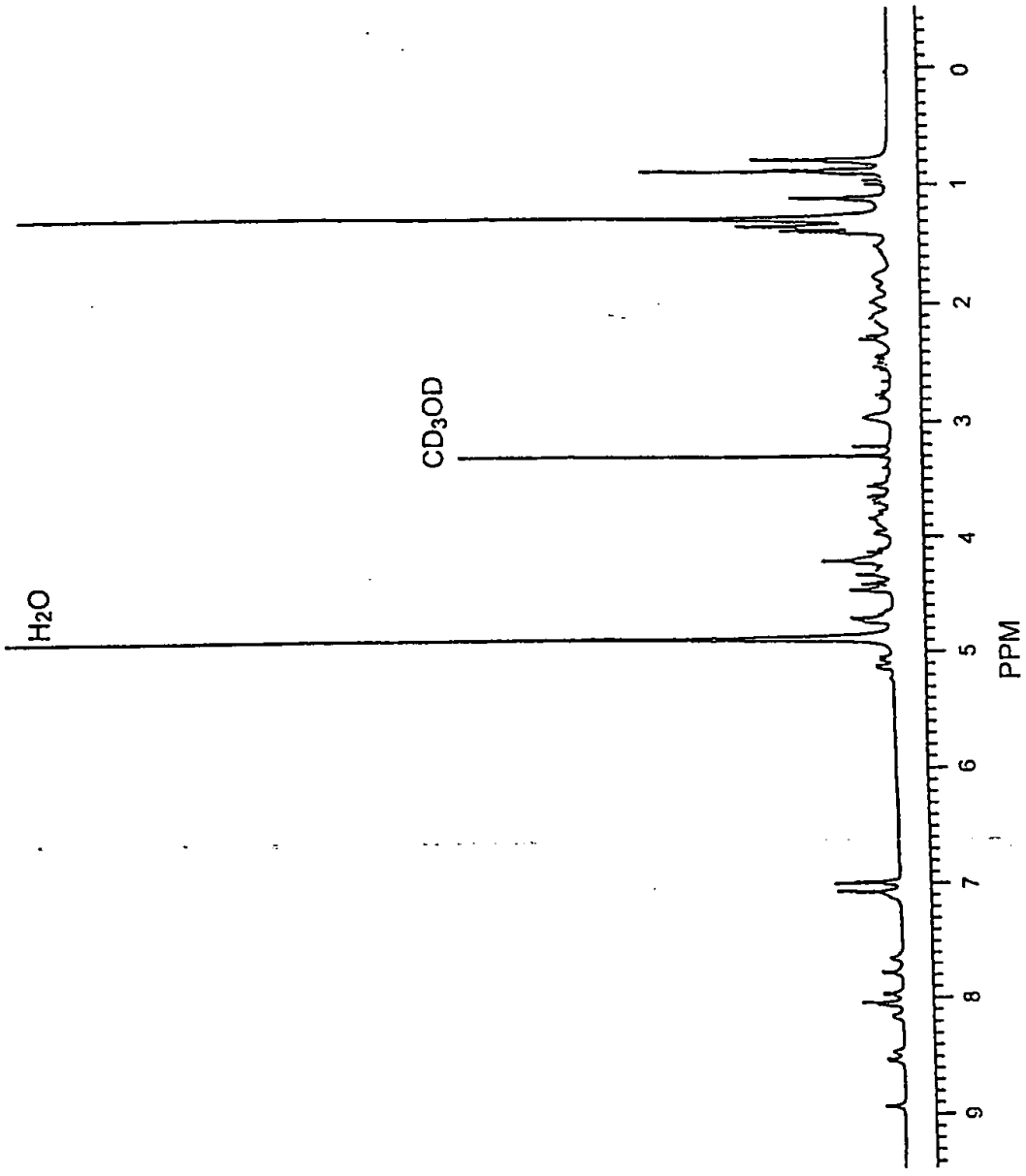
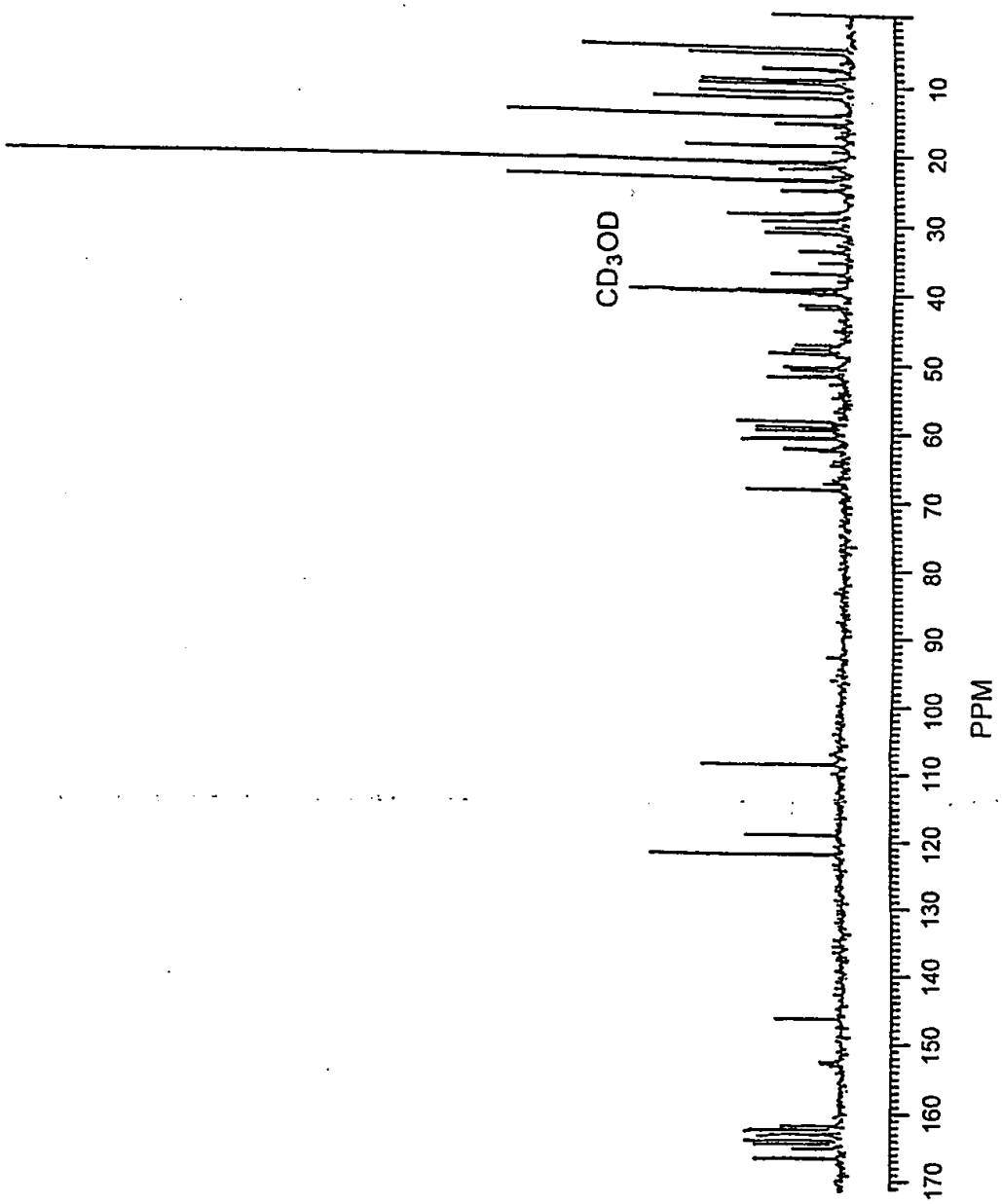


Fig. 4


CD3OD
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
PPM

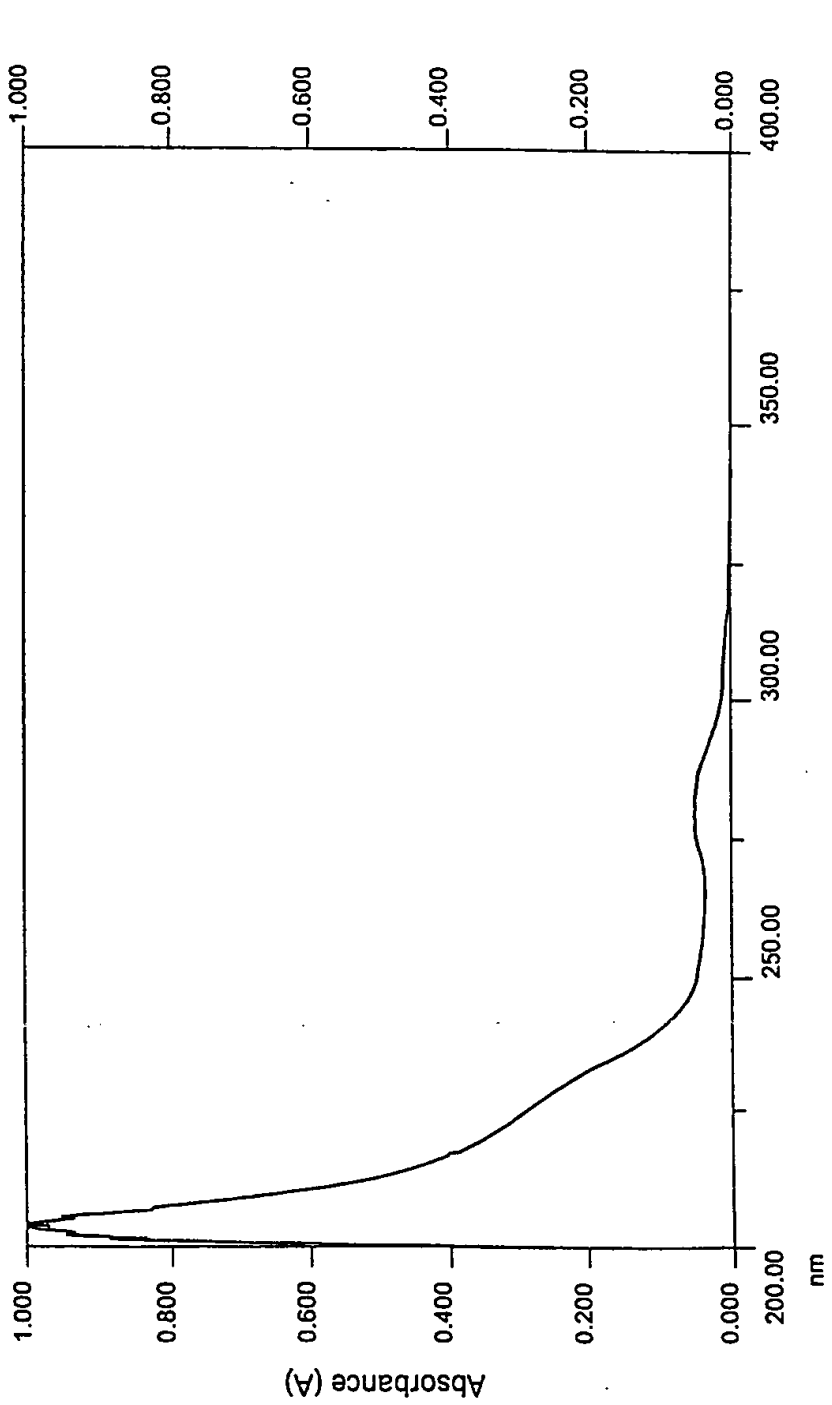

A
1.000
0.800
0.600
0.400
0.200
0.000
400.00
350.00
300.00
250.00
200.00
nm
Absorbance (A)

Fig. 5

Fig. 6

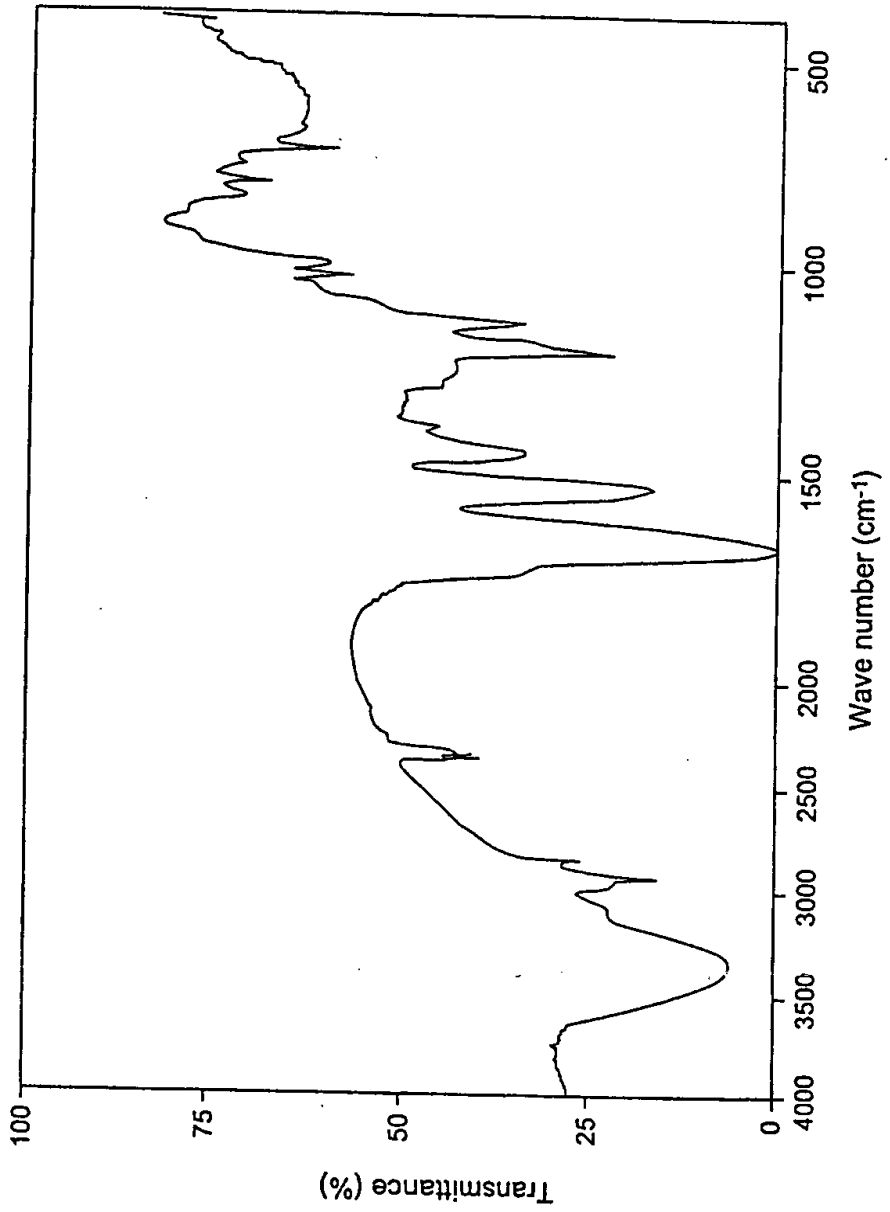
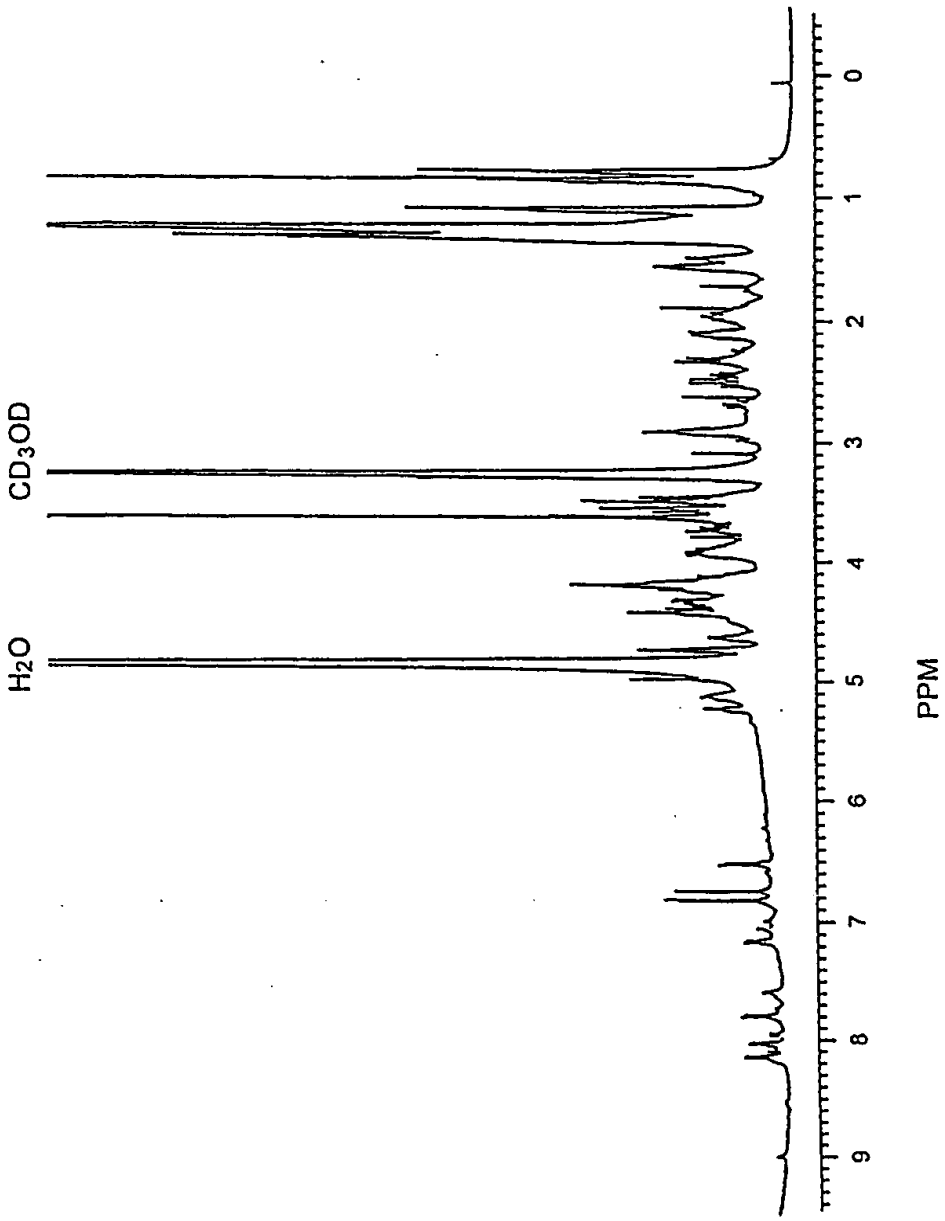

Transmittance (%)
100
75
50
25
0
4000
3500
3000
2500
2000
1500
1000
500
Wave number (cm-1)

Fig. 7



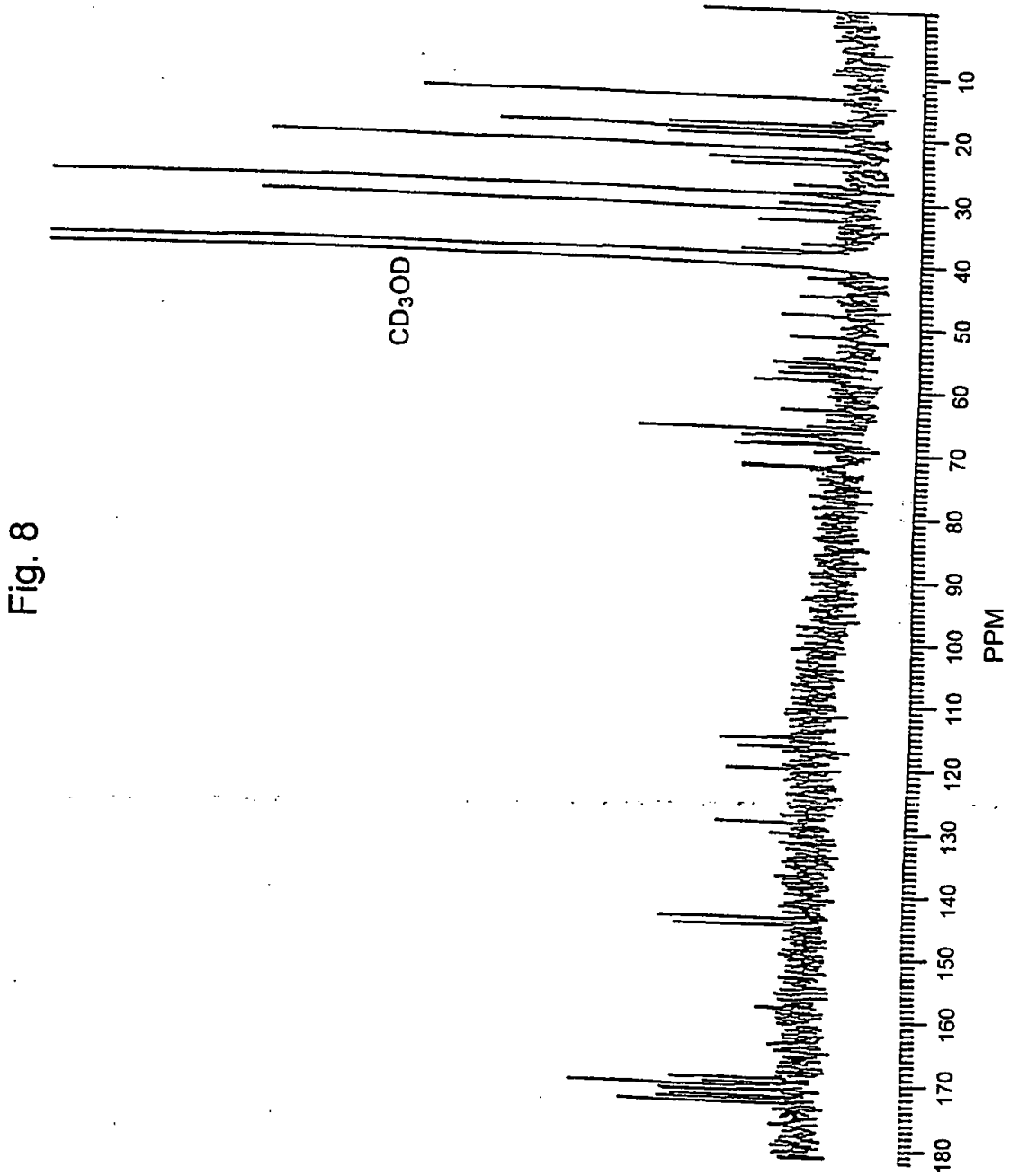

CD_3OD
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
PPM

Fig. 8

Fig. 9

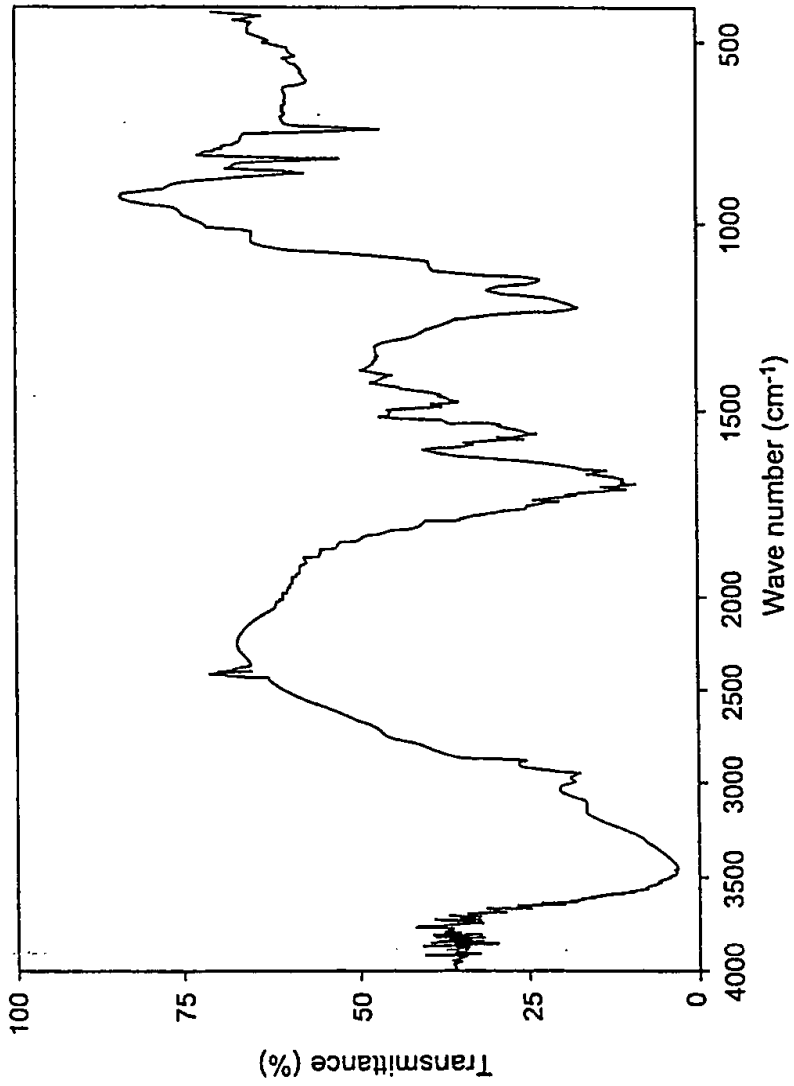

Transmittance (%)
100
75
50
25
0
4000
3500
3000
2500
2000
1500
1000
500
Wave number (cm-1)

Fig. 10

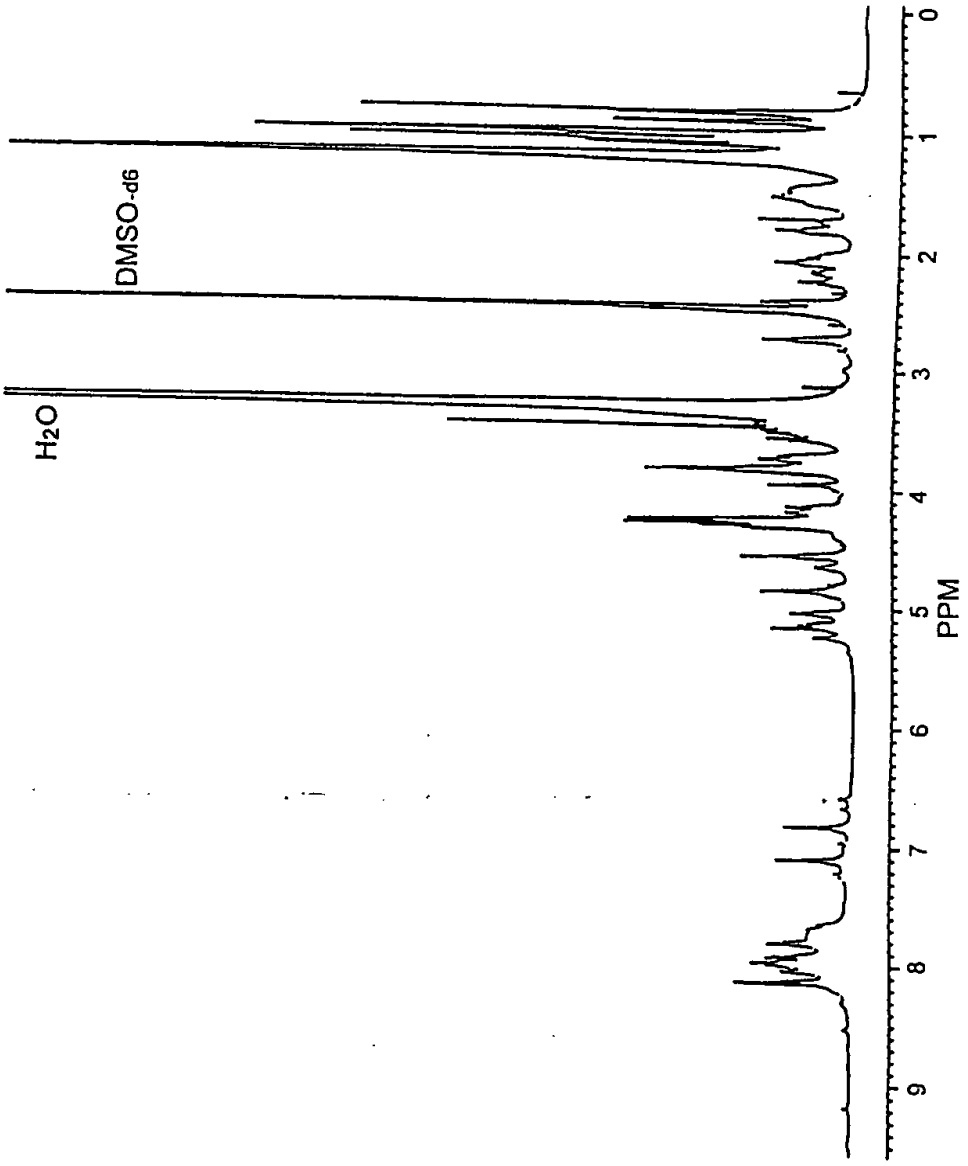
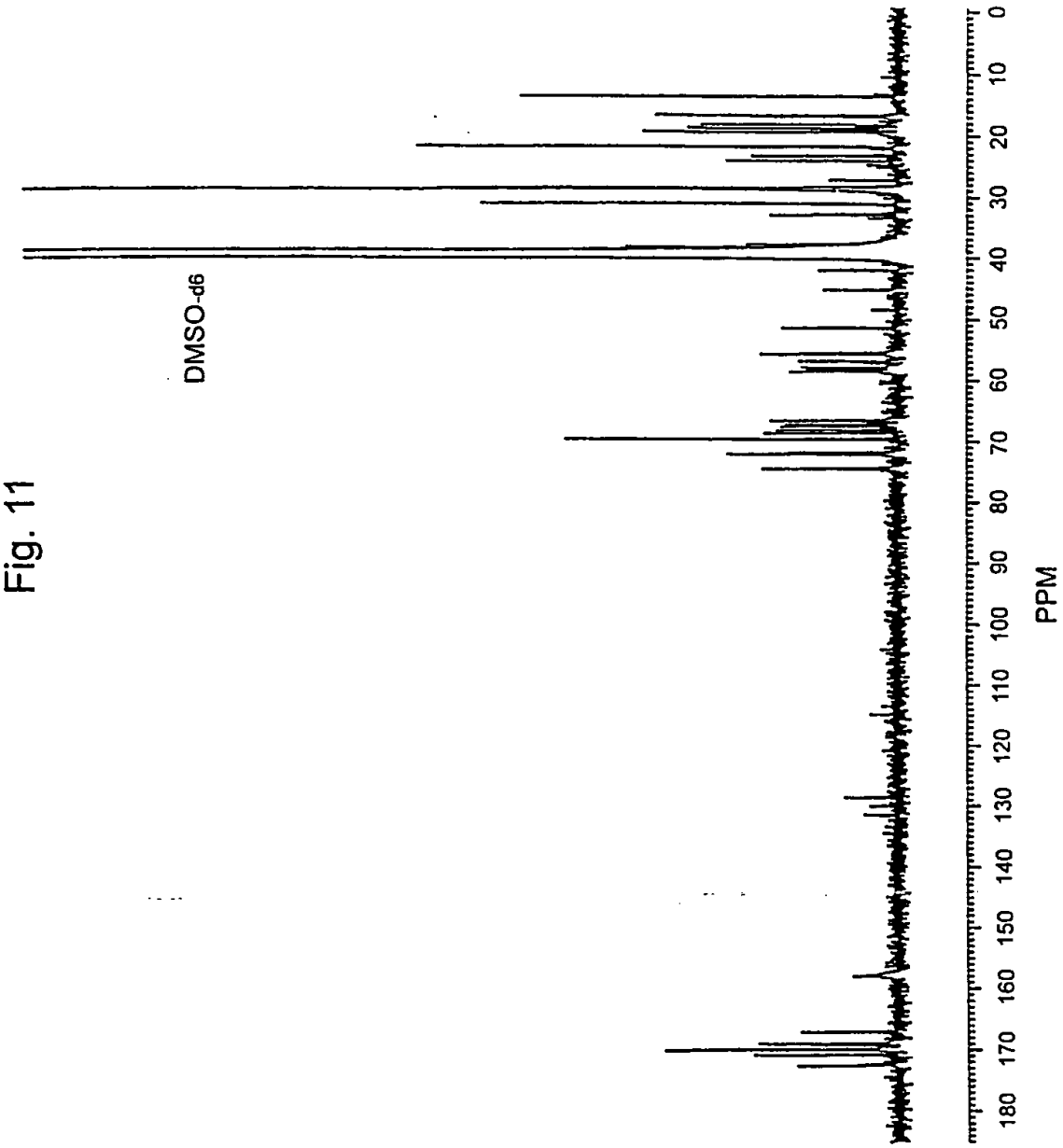


Fig. 11


DMSO-d6
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
PPM

INTERNATIONAL SEARCH REPORT

Inte ional Application No
PCT/EP 99/05235

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K7/56 C07K7/08 A61K38/10 A61K38/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 30399 A (HASHIMOTO MICHIZANE ;SHIGEMATSU NOBUHARU (JP); HASHIMOTO SEIJI (JP) 3 October 1996 (1996-10-03) the whole document	1-24,28, 29,31-33
A	EP 0 584 360 A (FUJISAWA PHARMACEUTICAL CO) 2 March 1994 (1994-03-02) cited in the application the whole document	
A	PATENT ABSTRACTS OF JAPAN vol. 1997, no. 11, 28 November 1997 (1997-11-28) & JP 09 176189 A (FUJISAWA PHARMACEUT CO LTD), 8 July 1997 (1997-07-08) abstract	

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 November 1999	08/11/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Groenendijk, M

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

.rnational application No.
PCT/EP 99/05235

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim 31 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte 'onal Application No
PCT/EP 99/05235

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9630399 A	03-10-1996	EP 0817796 A	14-01-1998
		JP 11505208 T	18-05-1999
		US 5952299 A	14-09-1999
EP 0584360 A	02-03-1994	JP 4335891 A	24-11-1992
		JP 5112599 A	07-05-1993
		AU 652639 B	01-09-1994
		DE 69217936 D	10-04-1997
		DE 69217936 T	19-06-1997
		US 5446022 A	29-08-1995
		US 5547934 A	20-08-1996
		AT 149521 T	15-03-1997
		AU 1740492 A	21-12-1992
		CA 2102705 A	10-11-1992
		HU 69150 A	28-08-1995
		IL 101717 A	08-12-1995
		JP 10045617 A	17-02-1998
		WO 9219648 A	12-11-1992
		JP 2661367 B	08-10-1997
		MX 9202145 A	01-11-1992
		HU 9500360 A	28-09-1995
JP 09176189 A	08-07-1997	NONE	